

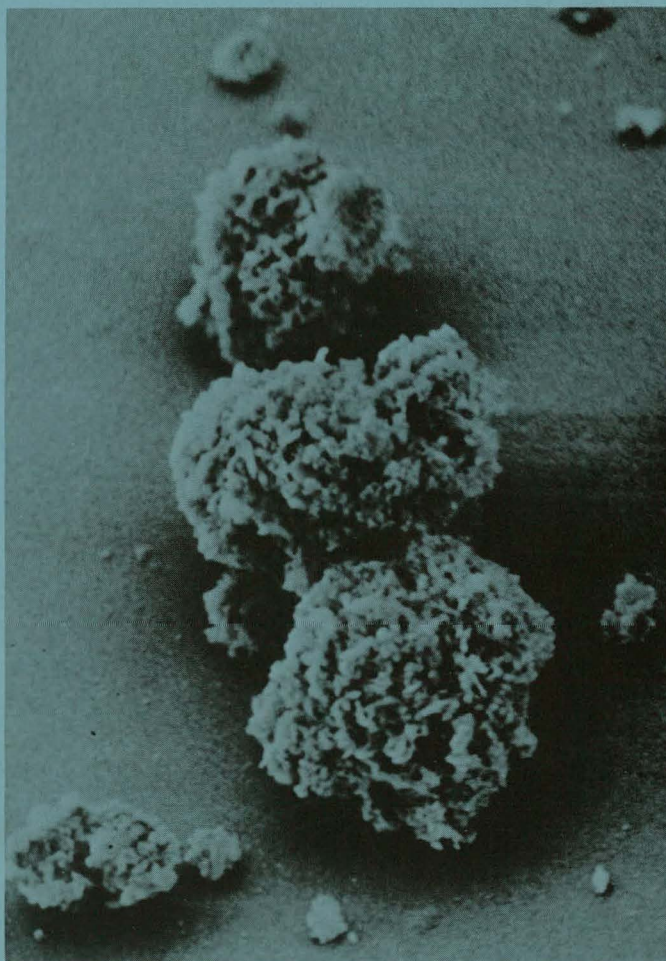
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**DIVISION OF BIOLOGICAL
AND MEDICAL RESEARCH**

MASTER

**Annual Report
1978**



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ARGONNE NATIONAL LABORATORY, ARGONNE, ILLINOIS

**Prepared for the U. S. DEPARTMENT OF ENERGY
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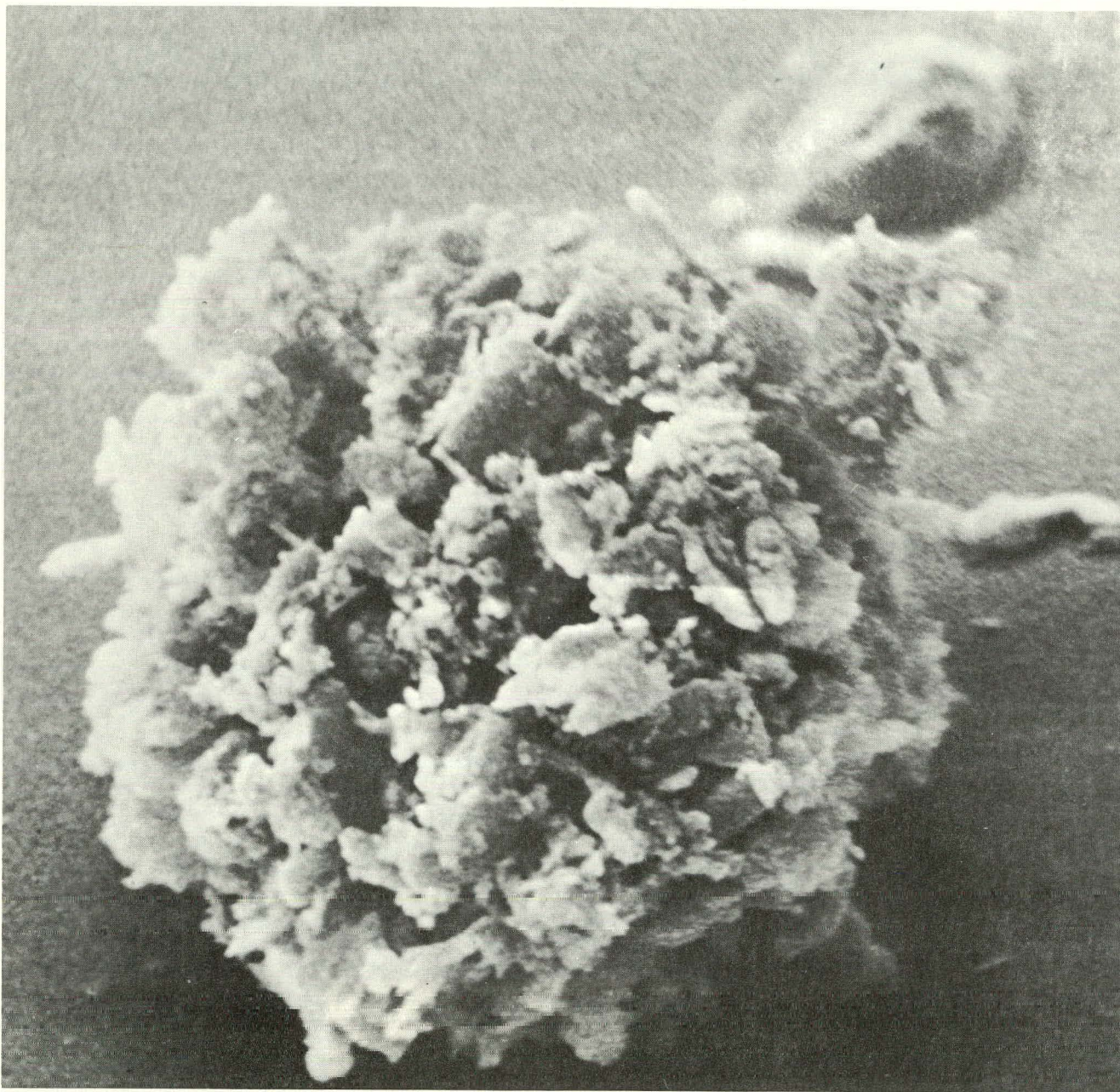
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Preceding Report

ANL-78-90



COVER

FLY ASH PARTICLES FROM FLUIDIZED BED COMBUSTION OF COAL, REAEROSOLIZED FOR CONTROLLED EXPOSURES TO EXPERIMENTAL ANIMALS (SEE PAGE 41). THE REPRESENTATIVE PARTICLE ABOVE HAS A DIAMETER OF 13.5 μm . SCANNING ELECTRON MICROGRAPHS TAKEN BY F. R. KIRCHNER.

ABSTRACT

The research during 1978 in the Division of Biological and Medical Research, Argonne National Laboratory, is summarized. Studies related to nuclear energy include responses of beagles to continuous low-level ^{60}Co gamma radiation, and development of leukemic indicators; comparison of lifetime effects in mice of low-level neutron and ^{60}Co gamma radiation; genetic effects of high LET radiations; and metabolic and therapeutic studies of heavy metals. Studies of nonnuclear energy sources deal with characterization and toxicological evaluation of effluents of fluidized bed combustion and coal gasification; electrical storage systems; electric fields associated with energy transmission; and development of population projection models and assessment of human risk. Basic research studies include fundamental structural and biophysical investigations; circadian rhythms; mutagenesis in bacteria and mammalian cells; cell killing, damage, and repair in mammalian cells; carcinogenesis and cocarcinogenesis; the use of liposomes as biological carriers; and studies of environmental influences on life-span, physiological performance, and circadian cycles. In the area of medical development, proteins in urine and tissues of normal and diseased humans are analyzed, and advanced analytical procedures for use of stable isotopes in clinical research and diagnosis are developed and applied. The final sections of the report cover support facilities, educational activities, the seminar program, staff talks, and staff publications.

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1. DIVISION DIRECTOR'S INTRODUCTION

This report is concerned with the progress of research in the Division of Biological and Medical Sciences in the calendar year 1978. The individual sections are organized into the four major activities of the Division. These activities are concerned with studies of the biological effects of nuclear energy technology; biological aspects of alternate energy sources, primarily coal; basic biomedical science; and development of medical technologies. The first seventeen sections present the activities of each of the research groups within the Division (Figure 1.1); in two cases, the work of a group is presented in two sections. Collaborations among groups are frequent, as indicated by cross references in the text and by the authorship of the individual research reports. Collectively, the introductions to the research sections provide an overview of the goals and accomplishments of the Divisional programs.

Finally, the last five sections of this report indicate the range of other activities in the Division. They present the major support facilities, the varied graduate and undergraduate level educational activities, the formal and informal seminars series in the Division, staff seminars and presentations at scientific meetings, and a list of staff publications appearing during the calendar year 1978.



Douglas Grahn, Director
Division of Biological and
Medical Research

9/78

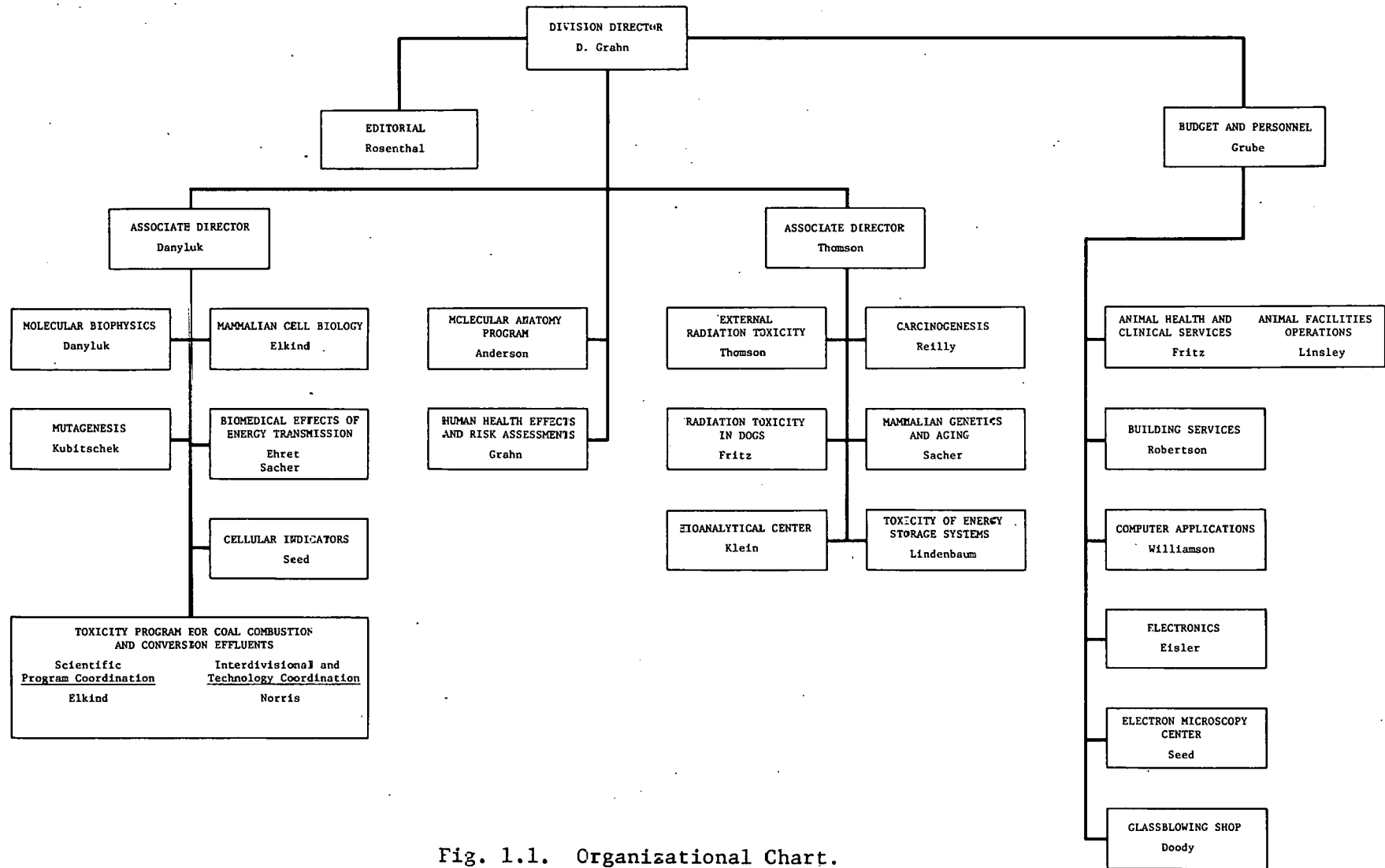


Fig. 1.1. Organizational Chart.

2. RADIATION TOXICITY IN DOGS

INTRODUCTION

Thomas E. Fritz

The basic objective of this study is the generation of data that will be useful for extrapolation to man on the late effects of low doses of ionizing radiation in a large, long-lived experimental animal. Lacking controlled data from exposed humans, particularly data on effects of low exposures after long time periods, it is necessary to use selected animal species and to search for biologically significant end points. Where interspecies differences are found, it is important to seek an explanation that can be expressed as a single unifying concept of predictive value for man. It is essential to measure the effects in a larger, long-lived animal because of the need for measuring longitudinal, sequential clinical responses.

The specific goals and areas of investigation within this broad objective can be summarized as follows:

- 1) To determine the influence of three major factors of exposure--(a) the daily irradiation exposure rate, (b) the accumulated exposure to irradiation, and (c) the influence of age at the time irradiation is initiated.
- 2) To provide data for estimates of radiation-specific excess mortality in the dog in order to allow interspecies comparisons to existing rodent data and to provide for extrapolation to man.
- 3) To provide base-line data and specimens to define the pathogenesis of leukemia and to study the mechanisms of radiation leukemogenesis.

Continuous or duration of life exposures, unlike single-dose or fractionated exposures, result in life shortening that is highly reproducible and has been found to take the mathematical form of an increase in the Gompertz slope relation. In laboratory rodents where adequate data are obtainable, the function has a well-defined slope, and at exposures between 25 and 125 R/day the Gompertz slope varies as the square of the daily exposure rate whereas at daily exposures below 25 R/day the trend of the slope changes to one that varies as the first power of daily exposure rate as determined by total dose.

The rate-squared slope (exposures of 25-125 R/day in mice) has been demonstrated only for rats and mice and has not been tested in longer lived species. This situation was a major consideration in the design of our study of continuous irradiation in beagles.

This research is being approached through a series of experiments that utilize protracted whole-body irradiation (22 hours/day, 7 days/week) from ^{60}Co gamma ray sources in three specially built exposure rooms. These experiments are characterizing the biological responses of beagles exposed at various exposure rates (1) until they die (beginning as young adults), (2) until they accumulate predetermined total doses of irradiation, and (3) during various stages of development and growth including fetal life.

Whole-body irradiation from ^{60}Co gamma rays was selected in order to simulate the effects of protracted irradiation from either (1) radionuclides deposited internally as a result of accidents with nuclear technologies or from weapon testing, or (2) external irradiation from increased ambient background from similar episodes. The dosimetry of such ^{60}Co exposures is relatively uncomplicated compared to that from deposited radionuclides and provides nearly uniform exposure to the whole body. The exposure rate and total dose, therefore, can be precisely controlled and varied and there is no excretion of radioactive materials.

In preliminary reports (e.g., Norris, W. P., and T. E. Fritz, in Radio-biology of Plutonium, Eds. B. J. Stover and W. S. S. Jee, Univ. of Utah Press, Salt Lake City, 1972, pp. 243-260; Fritz, T. E., et al. Late Biological Effects of Ionizing Radiation, Vol. II. IAEA International Symposium on the Late Biological Effects of Ionizing Radiation, Vienna, March 13-17, 1978, International Atomic Energy Agency, Vienna, 1978, pp. 71-82), the interactions of dose rate and total dose in determining tissue reactions to continuous exposure of the dog to whole body irradiation were discussed. The data demonstrated that over the range of exposure rates of 5 R/day to more than 100 R/day, the target organ is the hematopoietic system and the causes of death are related to the square of the dose rate. From the data then available it was decided to initiate a study at lower exposure rates designed to establish whether the change to unit slope would hold true in dogs, which have a mean life-span ~ 7 times that of laboratory mice, and are ~ 3.5 times more sensitive to acutely lethal exposures of ^{60}Co γ -irradiation. On the basis of a detailed analysis of the existing dog data at exposures of ≥ 5 R/day it was shown that significant mortality data could be obtained with groups of 80, 40, and 40 dogs at exposure rates of 0.4, 1.0, and 2.5 R/day, respectively, and these studies have been initiated.

The need to compare the effect of protracted irradiation given at various exposure rates to that given to death is also an important objective that developed early in these studies. This objective is being approached by exposing dogs in the gamma field until the exposure reaches either 600 or 1400 R at exposure rates of 35, 17, 10, or 5 R/day, 200 R at 17, 10, or 5 R/day, or 4000 R at 10 R/day.

As a result of the earlier studies at dose rates of 5 R/day and above, where leukemia was the most significant end point, an expanded collaborative study on the mechanisms of leukemogenesis was initiated (see Section 3).

REGULAR STAFF

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Kaspar, Lillian V. (Scientific Assistant)
Keenan, William G. (Scientific Associate)
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Poole, Calvin M. (Veterinarian)
Sanderson, Margaret M. (Scientific Associate)
Seed, Thomas M. (Assistant Biologist)
Tolle, David V. (Scientific Associate)

RESPONSES OF YOUNG ADULT BEAGLES TO CONTINUOUS EXPOSURE TO ^{60}Co GAMMA RAYS

T. E. Fritz, T. M. Seed, C. M. Poole, L. S. Lombard, D. V. Tolle,
W. G. Keenan, J. M. Angerman, S. M. Cullen, D. E. Doyle, L. V. Kaspar,
N. D. Kretz, P. H. Polk, and M. Sanderson

During the past year we have completed the entry of dogs into the lower exposure rate study. A total of 230 dogs includes 92 irradiated at 0.4 R/day and 46 each at 1.0 R/day, 2.5 R/day, and unirradiated controls. Only one dog has died but the death is not experimentally significant because it was caused by a parvovirus infection also seen in controls. To this time, those dogs irradiated for ~ 1000 days are showing a dose rate dependent depression of mean circulating platelet and total leukocyte numbers, while erythrocytes are largely unaffected. At 2.5 R/day the depression is obvious, while at 1.0 R/day it is of questionable significance when compared to controls. The values in dogs irradiated at 0.4 R/day are not different from control values. These results demonstrate that significant hematopoietic damage is occurring at 2.5 R/day and suggest the bone marrow aplasia or myeloproliferative disorders may result at higher exposure rates.

Six males at each exposure rate and six controls selected for sequential evaluation of the effects on spermatogenesis have now been followed for ~ 400 days. The dogs being irradiated at all three exposure rates are showing significant reductions in sperm counts. Those irradiated at 2.5 and 1.0 R/day have become essentially aspermic. Dogs irradiated at 0.4 R/day have sperm counts $\sim 10\%$ of preirradiation values and there are associated increases in abnormal morphology and reductions in live-dead ratio and motility. Thus we have already determined that reproduction cannot occur in the presently available gamma field dose rates because of the sensitivity of the male gonad.

INFLUENCE OF DOSE RATE AND TOTAL DOSE ON LATE EFFECTS OF TERMINATED EXPOSURES GIVEN YOUNG ADULT BEAGLES

T. E. Fritz, T. M. Seed, C. M. Poole, L. S. Lombard, D. V. Tolle,
W. G. Keenan, J. M. Angerman, S. M. Cullen, D. E. Doyle, L. V. Kaspar,
N. D. Kretz, P. H. Polk, and M. Sanderson

These studies continue to focus on the late effects occurring in dogs given total exposures between 600 and 2000 R at rates between 5 and 35 R/day as described earlier in this and in previous reports. Of the 357 dogs given terminated exposures, 193 lived more than 100 days following termination of irradiation and are being followed as chronic survivors and observed for late effects. A total of 52 of the chronic survivors have died to this time. Because of the schedule by which dogs were staged into the gamma field, survival time to date varies considerably. Some dogs have been observed as long as 3500 days after exposure while others have been out of the gamma field only 500 days. In addition to 9 hematopoietic tumors (leukemias and lymphosarcomas), there have been a variety of other malignancies including 8 mammary carcinomas.

From these interim data it is difficult to speculate on dose or dose rate effects except to say that 4 of the leukemias have occurred in the group given 4000 R at 10 R/day which is the highest total dose given to a group.

The minimum terminated exposure to produce myelogenous disorders still remains 1700 R, and the mean induction time is \sim 400 days. The incidence of myelogenous leukemia appears to decrease rapidly in dogs that survive beyond this time and, as in dogs irradiated until death, appears related to the recovery of the bone marrow--in this case, after termination of irradiation.

The large number of mammary malignancies, 8 among 21 female decedents, is significantly greater than in the colony control population where there have been 3 among 35 female decedents. A total of 6 nonmalignant mammary tumors in the dogs given terminated exposures is not appreciably different from the 11 in the control population. These data suggest that there is a higher frequency of total mammary tumors in these irradiated dogs and that the increase results from the malignant forms. It will be interesting and important to see whether these interim conclusions will be substantiated as the remaining dogs age and die. Subsequent to the early wave of myelogenous leukemias described above and the other soft tissue tumors, there were no other deaths that can be related to the irradiation.

EFFECT OF RADIATION AND RADIATION DOSE RATE ON BEAGLES IRRADIATED DURING FETAL LIFE

T. E. Fritz, T. M. Seed, L. S. Lombard, C. M. Poole, D. V. Tolle,
W. G. Keenan, D. E. Doyle, P. H. Polk, and M. Sanderson

As discussed in our previous reports, continuous irradiation given during fetal life produces discrete effects that are dose rate dependent and uniquely different (except for aspermia) from effects on adults. The most obvious is the failure of the adult gonads to produce gametes, thus resulting in sterility in both sexes. There are distinct differences in the sensitivities of the two sexes that are dependent on the dose rate given the fetus; oogenesis fails in the adult female given 5 R/day during fetal life, while spermatogenesis proceeds in the adult male at 5 R/day but not 10 R/day. Although normal in most other respects, both sexes also attain a size significantly less than normal in adult life. The lack of anomalies or birth defects has also been a significant finding in dogs given continuous irradiation during gestation.

A total of 62 adult dogs irradiated during fetal life is now surviving and being observed for late effects at ages between 500 and 2900 days of age. There have been eleven deaths in dogs surviving the neonatal periods. Two of these decedents, both irradiated at 10 R/day, died of renal disease at 918 and 1168 days of age. Another, irradiated at 5 R/day, died of a malignant lymphoma (lymphosarcoma) at 2561 days of age. These are the only deaths that appear related to the radiation exposure during fetal life. The remaining dogs appear normal except for the reproductive effects described above and their smaller than normal size.

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3. CELLULAR INDICATORS

INTRODUCTION

Thomas M. Seed

This new program, formed as an outgrowth of the Radiation Toxicity in Dogs Project, has three major research goals: (1) characterization of pathological progression in leukemia induced in dogs by ^{60}Co or benzene; (2) development of sensitive preclinical cellular predictors of impending disease; and (3) development and use in vitro of hemopoietic cell cloning methods for the clarification of the mechanisms of the hemotoxic responses and for the practical and rapid in vitro assessments of hemotoxic potential of radiation, benzene, and other potentially troublesome industrial or environmental pollutants.

Relative to the in vivo studies, the experimental approach has been to assess serially phase-related changes within the hemopoietic system of exposed animals. Work during the past year continues to support and to strengthen the concept that preleukemia is characterized by a progressive aberrant expansion of the granulocyte reserves and the committed stem cell compartments of the marrow. These early preclinical changes occur without the full complement of cytological cellular abnormalities that characterize overt leukemia. Further work in this area promises to identify specific cellular events in the pathological progression of induced leukemia.

The studies, as outlined, are based on the premise that leukemia induction is a progressive process with identifiable preclinical stages and that the process involves the selection and proliferation of aberrant hemopoietic clones. The developmental work on the in vitro cloning methods assumes that the pluripotent stem cell and its early commitment phases of differentiation are the basis of toxicant-induced hemopoietic dysfunction. Our efforts to identify, by immunological techniques, pluripotential hemopoietic stem cells should prove useful in assessing change in numbers and function of these key regulating cells during preclinical phases (see the report by Buchholz and Seed). The eventual application of the high resolution fluorescent labeling techniques to scanning electron microscopy (see the report by Oldfield et al.) will support our efforts to study stem cell function.

Several investigations by outside collaborators continue to assist the efforts to elucidate pathological mechanisms. These studies include: correlative studies of histocompatibility type (DLA) and proneness to radiation-induced marrow aplasia or leukemia (Drs. F. Wilson and K. Stitzel, University of California, Davis); sequential examination of serum profiles of suspect-preleukemic dogs (Dr. F. Quimby, Tufts University); and continued attempts to

detect oncornavirus by molecular probe methods designed to complement our direct electron microscopic searches (Dr. G. Todaro, National Cancer Institute).

We were pleased to have had Dr. Theodore Fliedner of the University of Ulm, (West Germany) for several days in our laboratory. In addition, Dr. D. Oldfield (Biology Department, DePaul University, Chicago) and Dr. J. R. Seed (Texas A & M University) were Faculty Research Participants in our laboratory. Dr. Oldfield is developing scanning electron microscopic luminescence techniques and Dr. Seed is studying tumor progression-regression as modulated by microbial infections.

REGULAR STAFF

Kaspar, Lillian V. (Scientific Assistant)
Seed, Thomas M. (Assistant Biologist)

TEMPORARY STAFF DURING 1978

Buchholz, Donna M. (Postdoctoral Appointee)

PRECLINICAL AND CLINICAL PHASES OF LEUKEMIA INDUCTION BY RADIATION

T. M. Seed, T. E. Fritz,¹ D. V. Tolle,¹ L. V. Kaspar, C. M. Poole,¹
 D. M. Buchholz, S. M. Cullen,¹ D. E. Doyle,¹ P. H. Polk,¹ J. M. Angerman,¹
 W. G. Keenan,¹ M. Sanderson,¹ and P. C. Brennan²

Previous studies have shown that under the condition of continuous, whole-body gamma (⁶⁰Co) irradiation at the dose rate of 10 R/day the incidences of aplastic anemia and myelogenous leukemia in beagles both approached 50%. A longitudinal study of the irradiated beagle's hemopoietic system is being carried out with the intent to localize structural and functional lesions and clonal aberrancies. The identification of specific hemopoietic lesions during preclinical phases should aid us in our prognostication of impending leukemia in irradiated dogs and perhaps in man.

To date, 30 dogs (4 replicate groups of 6 experimental animals, plus 6 controls) have been assigned to the current longitudinal leukemogenic studies and as of January 1979 are from 220 to 1027 days into the experiment after accumulated total gamma radiation doses of from 2200 to 10,270 R (at 10 R/day). Of 24 irradiated dogs, a total of 14 deaths have occurred resulting from aplastic anemia and two dogs have died (mean time of 455 days) with myeloproliferative syndromes characterized histopathologically as myelomonocytic leukemia and myelofibrosis. Six of the eight animals still alive are in suspected preleukemic states.

We presently recognize four distinct preclinical phases in leukemia development: (I) progressive development of severe leukopenia and thrombocytopenia, (II) partial hemopoietic recovery after 2000 R of accumulated exposure, (III) an equilibration of subnormal hemopoietic function, and (IV) a classical preleukemic phase. Overt leukemia is the fifth and last phase of the sequence which ends in terminal leukemia. Survivors of the hemopoietic crisis period (the terminal part of phase I), which predictably occurs between 200 to 300 days in the gamma field, show a broadly based recovery in hemopoietic function and have a high probability of developing myeloproliferative disease. The committed stem cell pool, following its initial (phase I) contraction to 10% of its pre-exposure size, slowly expands (phase II) from 30% to 40% of its original size and subsequently remains relatively stable (phase III) over several hundred days. Entry into phase IV appears to be characterized by a second, more rapid expansion of the granulopoietic stem cell compartment, for in several of our long-lived animals (i.e., > 800 days in the gamma field) the compartment has expanded beyond its pre-exposure size, but still remains smaller than in patently leukemic animals.

The sequential monitoring of the granulocyte reserves by the endotoxin stress assay has indicated corresponding time-dependent phase shifts, i.e., an initial contraction in phase I, followed by a small expansion in phase II to a subnormal level of approximately 25% of control value, and further expansion in late phase III and early phase IV.

¹Radiation Toxicity in Dogs Group.

²Toxicity Program for Coal Combustion and Conversion Effluents.

Correlated with the quantitative change in granulopoietic stem cells with time and accumulated dose of irradiation, are the qualitative, phase-related, changes in clonal growth properties. During the late part of phase I and early into the recovery phase (i.e., II), the developing colonies are atypical, i.e., small and loosely compacted. Growth in phase III is generally stronger; colonies are larger, more luxurious, and size/shape aberrancies are commonly noted. The latter growth patterns of phase III more closely resemble those of chronic leukemia, rather than the acute, blastic leukemia whose growth pattern is characterized by a "lawn-like" development of small clusters and single cells but with the formation of few or no colonies. Results of our cloning experiments also suggest that the absolute rate of spontaneous colony formation in the absence of specific canine serum factors (i.e., colony stimulating activity, CSA) increases during phases II and III.

The sequential change in CSA that regulates the proliferative and differentiative cycles of committed stem cells continues to be evaluated with time-of-irradiation and stage of pathological progression using normal, un-irradiated marrow stem cells as serum-responsive targets in the cloning assay. The serum CSA levels of dogs destined to radioaccommodate are initially highest early in phase I when the number of committed stem cells within marrow are at their nadir. There is a progressive decline in CSA activity which occurs concomitantly with the previously noted expansion of the stem cell pool. CSA activity during phase III is subnormal and remains so for several hundred days.

CHEMICAL LEUKEMOGENESIS, BENZENE INTOXICATION

T. E. Fritz,¹ T. M. Seed, S. Bourne,² A. Jirka,² N. D. Kretz,¹
D. V. Tolle,¹ M. A. Gabriel,³ and E. Lewandowski⁴

Benzene, a radiomimetic-like chemical, often produces upon prolonged exposure a marked suppression of bone marrow function and the occasional case of granulocytic leukemia, as the terminal pathological sequelae. Benzene is suspected to be leukemogenic in man based on epidemiological data; however, in contrast to the leukemogenic effects of ionizing radiation, the leukemogenic effect of benzene has not been conclusively documented in laboratory animals--hence the need for further toxicity testing.

¹Radiation Toxicity in Dogs Group.

²Analytical Chemistry Laboratory of the Chemical Engineering Division.

³Spring 1978 participant in the Undergraduate Honors Research Participation Program and participant in the 1978 Summer Research Institute in Biology, University of Dallas.

⁴Summer 1978 participant in the Undergraduate Honors Research Participation Program, University of Chicago.

During the past year an inhalation chamber designed to deliver an atmosphere containing 100-200 ppm of benzene and to handle approximately 50 mice was designed and built. Analytic procedures were developed for sampling and analyzing blood, by gas chromatography, for concentrations of benzene as low as 0.1 ppm. The latter is an important advancement, for it allows us to associate the variables of the route of exposure, the tolerated dosages, and blood and tissue concentrations relative to the extent of hemopoietic suppression.

The direct myelotoxic effect of benzene on cultured hemopoietic cells has been initially evaluated in vitro. We demonstrated a dose-dependent inhibition of stem cell (committed granulocytic-monocytic progenitors) cloning. At the concentration of 5000 ppm there was approximately 80% inhibition. The absolute viability of benzene-exposed cells, as assayed by trypan blue dye exclusion, appeared unchanged. Interestingly, the degree of inhibition was much more pronounced in the presence of autologous canine serum than in fetal calf serum; this would suggest a serum-specific sensitization.

DEVELOPMENT AND APPLICATION OF HEMOPOIETIC CELL CLONING METHODS AS GENERAL TOXICOLOGICAL ASSAYS

T. M. Seed, G. T. Chubb,¹ L. V. Kaspar, B. J. Wright,² and R. Ulrich³

This part of the Cellular Indicators program presently deals with an in vitro culture method in soft agar in which the proliferating and differentiating growth patterns of stem cells can be directly assessed following in vitro or in vivo exposure to the toxicant. With appropriate research and development, these in vitro assays might provide a rapid and sensitive assessment of hemotoxic potential. In essence, the key regulator cell of the hemopoietic system, i.e., the stem cell, would be used as an in vitro biological dosimeter to assess directly the hemotoxic (perhaps mutagenic) effects of ionizing radiation, benzene, and fluidized bed coal combustion effluents and thereby to reduce the need for extensive animal testing for hematological end points.

Time-dependent features of granulopoiesis in soft agar cultures have been studied over the past year. A degree of synchrony is displayed by cloned granulocytic cells in the terminal stages of colony development, and an incomplete maturation of granulopoietic cells is observed. In addition, we have successfully developed new ultrastructural methods that will enable topographical and spatial, as well as cytological, analyses of in vitro cloned cells.

¹Electron Microscope Center.

²Carcinogenesis Group.

³Thesis Parts Student, Western Michigan University.

Preliminary work to automate the colony counting part of the cloning assay using a modified image particle analyzer equipped with a TV camera and low power, wide angle lens to image and count colonies suggests that the system will enable us to evaluate, collate, and effectively store data concerning hemopoietic colony size, shape, and distribution.

ATTEMPTS AT IMMUNOLOGICAL IDENTIFICATION OF CANINE PLURIPOTENTIAL HEMOPOIETIC STEM CELLS

D. M. Buchholz and T. M. Seed

The intent of this study is to assess the functional change in pluripotent hemopoietic stem cells of dogs as a consequence of exposure to hemotoxic physicochemical agents, and to relate causally the induced cellular dysfunction to subsequent hemopathologies, e.g., aplastic anemia and leukemia.

An immunological approach is employed in attempting to identify both murine and canine pluripotent stem cells. It has previously been demonstrated in rodents that antisera directed to brain tissue identify stem cells (CFU-s) as measured in the Till and McCulloch spleen colony assay. Specificity of the antisera for the pluripotent stem cells has been verified by the inability to inhibit in vitro soft agar colony formation by the committed stem cells (CFU-c). We used the Till and McCulloch assay together with the in vitro soft agar assay to differentiate between the pluripotent (CFU-s) and the committed (CFU-c) stem cells. Antisera were prepared to both murine and canine brain tissue. Bone marrow cells removed from mouse femurs were treated in vitro as indicated in Table 3.1 and then injected into irradiated recipient mice for assay of spleen colony formation. Table 3.1 demonstrates the inability of the antisera-treated murine marrow cells to form splenic colonies. This inability demonstrates that the pluripotent stem cells (CFU-s) have been inhibited, and thus identified, by the antisera.

Table 3.1: Effect of Anti-Brain Sera on Mouse Spleen Colony Formation

Treatment of Marrow Cells ^a	CFU/10 ⁵ N.C. ^b
Hanks' balanced salt solution	41.67
Normal rabbit serum + C'	36.02
Rabbit anti-mouse brain serum + C'	4.57
Rabbit anti-dog brain serum + C'	6.57

^aTreatment consisted of a 45 minute incubation at 0°C with the designated serum followed by a 30 minute incubation with guinea pig complement (C') at room temperature.

^bColony forming units per 10⁵ nucleated cells.

Treatment of mouse bone marrow cells with either rabbit anti-mouse brain or rabbit anti-dog brain antiserum significantly decreased spleen colony formation (Table 3.1), either by suppressing proliferation of the stem cells or by inactivating the stem cells by cytolysis. By indirect immunofluorescence the fully absorbed antisera (i.e., against liver, spleen, thymus, and peripheral blood) specifically label a small lymphocyte-like subpopulation of both dog and mouse bone marrow cells. Our preliminary in vitro evidence using the soft agar assay indicates that the antisera specifically identify only pluripotential, not committed, stem cells. Further morphological identification of these immunologically identified cells will be carried out by electron microscopy employing ferritin-labeled antibody.

SPECTRAL ANALYSIS OF LUMINESCENCE FROM BIOLOGICAL STAINS USING THE SCANNING ELECTRON MICROSCOPE

D. G. Oldfield,¹ G. T. Chubb, and T. M. Seed

The purpose of this study is to develop apparatus and techniques for obtaining luminescence of several biological stains (dyes) at high magnification using the scanning electron microscope (SEM). A microanalytical method of this type would be a distinct improvement over existing methods since it would give simultaneously both the high spatial resolution afforded by the SEM and the high chemical resolution obtainable from luminescence spectra of known dye molecules. When the dye molecule used is such that it (or its conjugate) binds preferentially to one particular cell component (e.g., membrane glycoprotein, cytoplasmic carrier protein, chromosomal nucleoprotein, etc.), the method would be expected to provide a more accurate and more sensitive in vitro localization and quantitation of cell components than is feasible using conventional techniques. Thus, subtle cellular effects of toxicologic, mutagenic, or tumorigenic agents might be detected earlier and/or more sensitively than by methods currently in use.

The apparatus used has been described previously (Oldfield, D. G., et al., ANL-75-30, 1974, p. 98). For initial checks on the system, two types of size-graded fine sulfide particles (one exhibiting red fluorescence, the other exhibiting green fluorescence under optical excitation) were used. Also used in the initial work were the fluors anthracene, dimethylphenyl-oxazolyl benzene, and divinylbenzene-impregnated polystyrene spheres and nine different biological stains.

Luminescence spectra have been obtained from the above luminors in two different ways: (1) by setting the interference filter to several different wavelengths while the specimen field is being scanned, holding each wavelength setting for 20 seconds; and (2) by holding a single, constant wavelength setting while the entire specimen field is scanned. The first method is more rapid and can be particularly useful at high magnification when inhomogeneities

¹Faculty Research Participant, DePaul University, Chicago.

in specimen detail are expected to be negligible. The second method is more useful when only a few wavelengths are to be examined or when it is desirable to obtain color prints of the luminescence.

Our results to date indicate that appreciable differences in luminescence intensity exist between different luminors (judged on the basis of weight of luminor used). For example, the acridine orange specimen emitted relatively weakly while the fluorescein specimen emitted relatively strongly. The spectra obtained were in reasonable agreement with expectations from optically induced fluorescence as judged by simple qualitative evaluations of the optical densities obtained. The next problem to be addressed is that of detection sensitivity for dyes bound in cells.

4. NEUTRON AND GAMMA-RAY TOXICITY STUDIES

INTRODUCTION

John F. Thomson

The Neutron and Gamma-Ray Toxicity Program, which has as its objective the study of the late effects of low levels of ionizing radiation in experimental animals, consists of two subtasks, one concerned with observation of animals for their entire life-span and subsequent establishment of the causes of death, the other focusing on specific pathophysiologic effects of ionizing radiation on the structure and function of the cardiovascular system.

Both subtasks employ the same radiation sources (^{60}Co gamma rays and fission neutrons from the JANUS reactor), the same species of animals (Mus musculus, the B6CF₁ hybrid of the C57BL6 and BALB/c strains of the laboratory mouse; and Peromyscus leucopus, the white-footed mouse, descended from wild animals trapped on the Argonne site), and the same logistic support for dosimetry, data processing, and animal maintenance.

The radiation facilities also support numerous other programs in the Division; special mention should be made of Dr. Douglas Grahn's studies on Genetic Effects of High-LET Radiation (reported in Section 5, following), which seek to establish dose-response curves for several genetic end points over the same dose ranges for which we are measuring somatic effects.

At the present time, exposures of Mus musculus have been completed to provide dose-response curves, for all causes of death and for specific causes, under the following conditions:

- 1) Single neutron exposures 5 to 240 rads.
- 2) Single gamma-ray exposures, 90 to 788 rads.
- 3) Fractionated neutron exposures (24 fractions given at weekly intervals), 10 to 320 rads.
- 4) Fractionated gamma exposures, 206 to 5110 rads.
- 5) Weekly neutron exposures for duration of life, 0.67, 1.67, and 2.67 rads per fraction.
- 6) Weekly duration of life gamma exposures, 7, 17.4, and 31.9 rads per fraction.

We have also completed exposures of Peromyscus leucopus to single doses of neutron (20 to 160 rad) and gamma (90 to 417 rad) radiation. Although many Mus and Peromyscus are still alive, a number of conclusions can be drawn, which are not likely to change qualitatively, concerning the comparative effects of neutron and gamma radiation on life shortening; these are discussed in the first report.

The second report in the series deals with the acute toxicity of neutron and gamma radiation to Mus and Peromyscus. The latter is more resistant than Mus to gamma rays, but more sensitive to neutrons.

Then follows a status report on the effects of neutrons and gamma rays on the cardiovascular system. Previous studies of radiation effects on coronary arteries have been supplemented by electron-microscopic examination of renal arteries, which show a similar spectrum of degenerative changes.

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STUDIES OF LIFE SHORTENING

J. F. Thomson, F. S. Williamson, L. S. Lombard, K. H. Allen,
J. L. Hulesch, V. A. Ludeman, A. R. Sallese, and E. F. Staffeldt

Since the previous report (ANL-78-90), emphasis has been directed toward completion of various exposure series then under way; no new major experiments have been initiated.

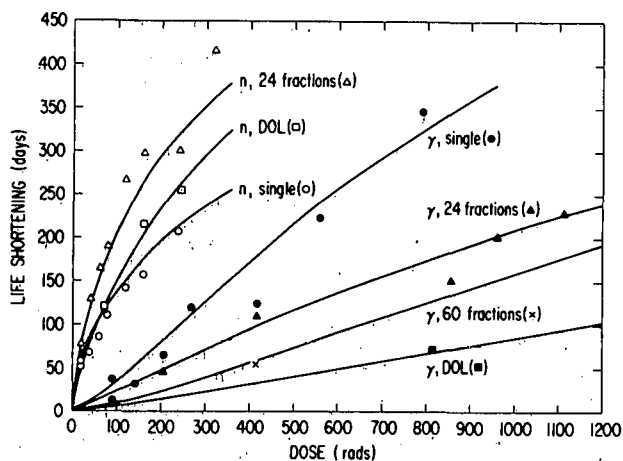


Fig. 4.1. Dose-response curves for male mice given single, fractionated, or duration-of-life (DOL) exposures of neutron or gamma radiation. Animals were 110 days old when irradiation was begun.

Figure 4.1 shows the life shortening produced in male B6CF₁ mice by neutrons and gamma radiation in various exposure modes (similar data, involving fewer data points, are available for female mice). The graph does not show the data for life shortening produced by exposure to fractionated gamma radiation at total doses greater than 1110 rads.

From these data a number of conclusions can be drawn, despite the fact that appreciable numbers of mice are still alive at the lowest dose levels:

1) Fractionation of gamma radiation results in a longer mean survival time than that observed when the same total dose is given in a single exposure; and the more extensively the dose is fractionated, the less effective is the total dose.

2) Fractionation of neutron radiation, on the other hand, produces an augmentation of life shortening that is statistically significant at high doses (> 100 rads); at lower doses, although the evidence for augmentation may not be convincing, there is at least full additivity.

3) Females are more sensitive than males to neutron but not to gamma radiation (data not shown).

4) Less life shortening is observed in animals exposed to single doses of neutron or gamma radiation at 194 and 278 days of age than at 110 days, the age at which the animals were exposed in most of the experiments (data not shown).

5) The single-dose RBE for JANUS fission neutrons relative to gamma rays varies inversely with the square root of the neutron dose over a range of 20 to 240 rads; projections from the present data suggest that at 1 rad the RBE will be about 40.

ACUTE RADIATION TOXICITY

J. F. Thomson, F. S. Williamson, J. L. Hulesch, G. L. Holmblad,
E. G. Johnson, Jr., and J. E. Trier

Since the first exposures of mice in the JANUS program were begun 8 years ago, we have observed a slight but perceptible increase in the life-spans of control animals, both males and females. We thought it desirable to remeasure the acute radiation toxicity of the B6CF₁ mice, not only for comparison with the 1971 determination (Ainsworth, E. J., et al., ANL-7770, 1970, p. 32), but also for anticipated comparison with mice to be used in future experiments involving rederivation of the breeder strains and maintenance of the mice in isolators. In addition, we have measured the 30-day LD₅₀'s for Peromyscus leucopus.

The data are shown in Table 4.1. The values calculated for the 30-day LD₅₀'s in the present experiment do not differ significantly from those reported by Ainsworth et al. (367 and 905 rads for neutron and gamma radiation, respectively) when the Ainsworth et al. values are corrected in light of dosimetric refinements. For P. leucopus we have confirmed their somewhat greater resistance than Mus to gamma rays, so that the RBE for acute toxicity is about 35% higher.

Note also that the slopes of both probit-dose lines for P. leucopus are only 40% as steep as the corresponding slopes for Mus. This observation reflects the considerably greater heterogeneity of the Peromyscus.

Table 4.1. Acute Toxicity of Neutrons and Gamma Rays

	<i>Mus musculus</i> ^a	<i>Peromyscus leucopus</i> ^b
Neutrons		
LD _{50/30} (rads) \pm SE	357 \pm 4	295 \pm 10
Slope (probits/rad) \pm SE	0.062 \pm 0.013	0.026 \pm 0.012
Gamma Rays		
LD _{50/30} (rads) \pm SE	923 \pm 9	1014 \pm 19
Slope (probits/rad) \pm SE	0.021 \pm 0.004	0.0082 \pm 0.0012
RBE	2.6	3.5
^a B6CF ₁ males, 122-129 days old.		
^b Males, 140-154 days old.		

CARDIOVASCULAR EFFECTS OF EXTERNAL RADIATIONS

S. P. Stearner, E. J. B. Christian, R. L. Devine, R. S. Skyer,¹ and
T. Riss²

Long-term cardiovascular changes were studied in the irradiated B6CF₁ mouse. Total-body radiation treatment included a single dose of 788 gamma rads or 80 or 240 neutron rads, or a fractionated dose (24 weekly fractions) of 823 or 2690 gamma rads or 20, 80, or 240 neutron rads. Comparisons of structural and functional changes continue to be emphasized. Ultrastructural observations (transmission electron microscopy) through 24 months revealed degenerative changes in the myocardium as well as in coronary arteries, aorta, and blood vessels of other tissues. Degeneration was generally more extensive after fractionated neutron irradiation than after comparable single doses. In

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² Participant in the 1978 Summer Research Institute in Biology, Illinois State University.

contrast, fractionation of the gamma dose reduced the resultant damage.

Damage to myofibers and capillaries of the heart was most severe at 30 to 90 days after a single exposure, but persisted above control levels throughout the 24-month observation period. Matrix accumulations (proteoglycans) and fibrosis were more prominent in neutron- than in gamma-treated animals; these conditions correspond to similar reactions following neutron doses in the therapy range reported in the clinical literature.

Degenerative changes in coronary arteries became prominent only after 6 to 12 months; at 18 and 24 months there was persistent and increased damage, especially after fractionated neutron treatment. Most severe damage was to medial smooth muscle, whereas endothelium appeared largely intact at later times. Evaluation of degenerative changes in renal arteries, as yet incomplete, indicates they are similar to those in coronary arteries, but are somewhat less severe in relation to radiation treatment. Noteworthy are clusters of abnormal smooth muscle cells that may represent abortive attempts at regeneration or remnants of medial cells displaced to the adventitia. The augmented effect of fractionated neutron exposures has relevance to the clinical use of fractionated high-LET radiations in the radiotherapy of tumors in humans.

Evaluations of late changes in blood pressure are of interest in view of our observations of late degenerative changes in arterial smooth muscle and elastica. Blood pressure measurements made to date with a pressure cuff developed (in collaboration with G. Svihla) for use on the mouse hind limb revealed no consistent change in mean systolic pressure in relation to age or radiation treatment. After irradiation, however, animals had higher values, and a fall in blood pressure was correlated with the near-terminal condition.

In conclusion, studies of structural damage in irradiated mouse arteries revealed unexpected sensitivity, especially to fractionated doses of fission neutrons. The greater the degree of coronary artery degeneration the greater was the extent of myocardial damage. Fractionation of the exposure augmented neutron dose effectiveness, whereas gamma-ray fractionations reduced the resultant damage.

5. GENETIC EFFECTS OF HIGH LET RADIATIONS

INTRODUCTION

Douglas Grahm

This series of studies was motivated by the observation that there were no experimental data upon which to make judgments about the potential genetic hazards of gonadal burdens of plutonium or the other transuranic elements. Genetic studies have been performed at two levels of ^{239}Pu , injected as single doses intravenously as the citrate into male mice. Dominant lethal mutation rate, chromosome translocation rate in spermatogonial cells, and the frequency of abnormal sperm have been measured in a comparative analysis of the effects of internally deposited plutonium alpha particles, and external radiation from fission neutrons and ^{60}Co gamma rays. These experiments are near completion and the results indicate that neutrons and alpha particles have approximately the same mutagenicity. They both have a greater mutagenicity than gamma rays, but the ratio of effectiveness depends upon germ cell stage, dose rate, total dose, and pattern of exposure. The minimum ratio of neutrons or alpha particles over gamma rays is about 5, for lethals induced in mature germ cells by single exposures, and the maximum effectiveness ratio is about 40, for the induction of reciprocal chromosome translocations following protracted low intensity exposures.

This effort led to a detailed study of these same genetic end points following extremely low single doses of neutrons (1 to 10 rads) as it became apparent that reliable dose-response data were not available for the conventional external radiation sources at low doses. For some end points significant responses are seen at doses as low as 1 to 5 rads. The low dose issue also arose as a result of two additional sets of observations: the reexamination of selected human data on low dose neutron leukemogenesis and the recurring experimental observations on mice that low neutron doses might have an unexpectedly high potential for inducing biological damage.

In the past year, we have also performed an initial study with ^{241}Am to determine whether this element would show the same pattern of gonadal uptake and distribution as ^{239}Pu , and whether deposition of americium would be associated with the same level of genetic damage per unit of injected dose.

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DOMINANT LETHAL MUTATIONS AND CHROMOSOME ABERRATIONS INDUCED IN MALE MICE BY INCORPORATED ²³⁹Pu AND EXTERNAL FISSION NEUTRON AND GAMMA IRRADIATION

D. Grahn, B. H. Frystak, C. H. Lee, J. J. Russell,¹ and A. Lindenbaum²

The genetic effects of gonadal burdens of ²³⁹Pu following intravenous injection of plutonium citrate at levels of either 5 or 10 μ Ci/kg have been compared over the reproductive lifetime of male B6CF₁ mice with the effects of external whole-body ⁶⁰Co gamma ray or fission neutron irradiation. Gamma irradiations were given as single, weekly, or continuous (22 hours/day) exposures and neutron irradiations were given as single or weekly exposures. Testicular ²³⁹Pu burdens remain unchanged at 0.05 percent of the injected dose for over 400 days and localize in the interstitial tissue and along the basement membrane of the spermatogenic tubules.

The dominant lethal mutation rate with alpha particle irradiation is 0.0064 ± 0.0011 mutations/gamete/rad. The average rate for postmeiotic germ cells is similar to this following single and weekly doses of neutrons. These rates are 5-fold greater than those following single or weekly gamma irradiation and more than 10-fold greater than the rate following continuous irradiation.

For the induction of reciprocal chromosome translocations, weekly neutron exposures are more effective than the same dose given as a single exposure, above ~ 20 rads, and the dose response is linear, compared to the nonlinear response after a single dose of neutrons. The translocation rate following continuous gamma irradiation is 0.175 ± 0.017 ($\times 10^{-4}$)/rad, which is about 1/10th the effect of single gamma irradiation and 1/40th the effect of weekly neutron exposures. The translocation yield shows no response to accumulating dose from plutonium alpha particles. The responses were above that seen following continuous gamma irradiation and below the response to neutrons. However, the average frequency of translocations is related to the measured gonadal burden. This finding that plutonium-induced genetic damage to the stem cell population does not follow a linear, cumulative dose-response function may be due to spermatogonial cell killing, to relocation of the plutonium away from the basement membrane, to the possible establishment of spermatogonial clones in radiation-free regions of the tubule, or to some combination of these factors.

There are dose and dose-rate dependent relationships for effects related to damage induced in spermatogonia just prior to meiosis and in all meiotic and postmeiotic cell stages; however, total dose accumulations are limited to the 4 to 6 week period of gamete development and maturation. The evidence supports the position that the majority of the dominant lethal mutations transmitted by males carrying plutonium burdens are induced in the meiotic and postmeiotic cell stages, not in the stem cell population. This is an important discrimination with regard to considerations of the potential genetic

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risks that might be associated with long-term body burdens of plutonium and the other transuranic elements, since it establishes that the mutagenicity of plutonium is about equal to that of high LET fission neutrons.

GENETIC EFFECTS OF LOW SINGLE DOSES OF NEUTRONS AND GAMMA RAYS

D. Grahn, B. H. Frystak, and C. H. Lee

This single dose experiment compares neutron dose levels of 1, 2.5, 5, 10, 20, and 40 rads with a limited gamma ray series of 22.5, 45, and 145 rads in ~100-day-old B6CF₁ male mice. Sampling statistics were established on the expectation of detecting significant ($P \sim 0.05$) changes at 2.5 to 5 rads and above so that the response to 1 rad could be judged for its conformance to predictions based upon the data at the higher doses.

There is evidence for a slight, and sometimes significant, excess of response to the neutron doses of 1 to 5 rads when compared to the expectations linearly projected from the higher doses. This excess response is seen for the average number of live fetuses per pregnant female, the incidence of preimplantation lethality, and the incidence of postimplantation loss. It is suggested in the data for numbers of chromosome translocations, but it is not seen in the data for abnormal sperm. At most, the excess effect over expectation is by a factor of two. These data are summarized in Table 5.1 and Figures 5.1 and 5.2.

Table 5.1. Summary of Responses to Low Single Doses of Fission Neutrons and ⁶⁰Co γ-Rays^a

Dose Level (rads)	Live Fetuses Per Pregnant Female ^b	Dom. Lethal Rate/Gamete		Abnormal Sperm %	Cells with Translocation %
		Preimp.	Postimp.		
Control	7.59 ± 0.15 (141)	.077 ± .008	.047 ± .006	2.37 ± .18	0.021 ± .021
Neutrons					
1	7.45 (242)	.071 (.60) ^c	.063 (.09) ^c	2.69 (.25) ^c	0.068 (.35) ^c
2.5	7.22 (172)	.099*	.069*	3.32**	0.265**
5	7.11 (133)	.104*	.073*	4.46**	0.373**
10	7.26 (66)	.089 (.40) ^c	.092**	7.17**	1.06**
20	6.64 (66)*	.132**	.152**	12.56**	0.60**
40	5.43 (58)**	.171**	.259**	24.92**	1.90**
γ-Rays					
22.5	7.29 (136)	.095 (.14) ^c	.068*	3.71**	0.216**
45	7.35 (66)	.104 (.08) ^c	.074*	6.88**	0.429**
145	6.44 (59)*	.106 (.07) ^c	.202**	16.82**	1.38**

^aMale B6CF₁ mice subjected to whole body exposure at 100-120 days of age. Dominant lethal mutations (preimplantation and postimplantation) and related data from the first five weeks of mating after exposure; abnormal sperm measured at weeks 5 and 6; translocations scored at weeks 5 to 7.

^bNumber of pregnant females in parentheses.

^cEstimated P value from χ^2 test; one degree of freedom for control vs. dose. * $P \leq .05$. ** $P \leq .01$.

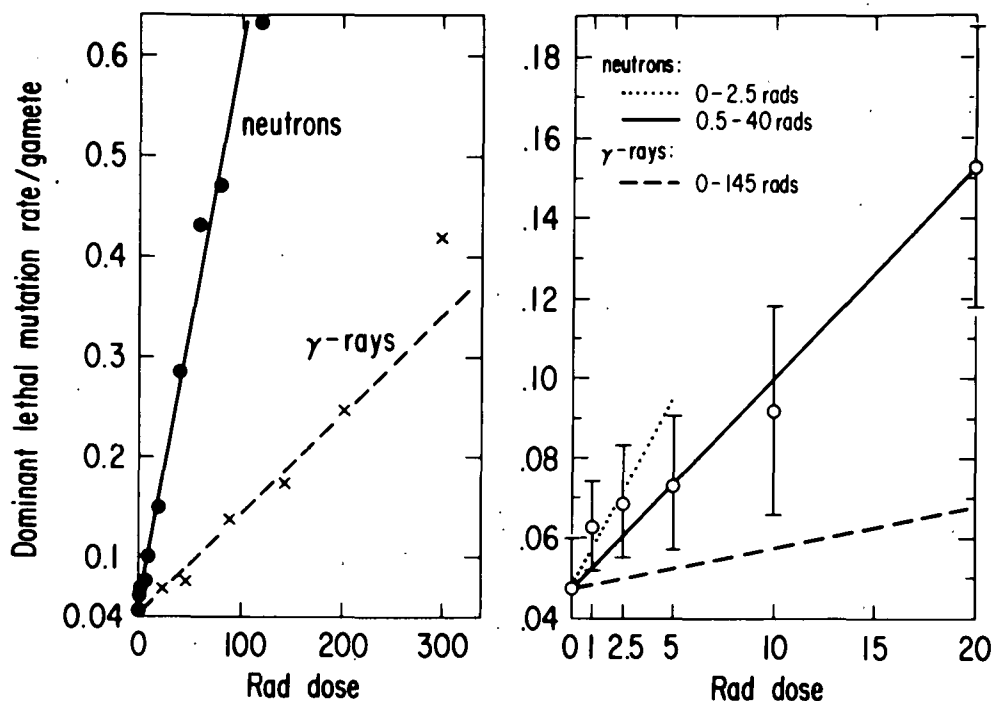


Fig. 5.1. Postimplantation dominant lethal mutation rate summed over the first 5 weeks after single exposure. Rates are based upon fetal survival and expressed as:

$$-\ln\left(\frac{\text{live fetuses}}{\text{total implants}}\right).$$

On the right-hand panel, mutation rate estimates for the identified portions of the data were 0.0096 ± 0.0023 (neutrons, 0-2.5 rads, three dose levels); 0.0052 ± 0.0001 (neutrons, 0.5-40 rads, five dose levels); 0.0010 ± 0.0001 (gamma rays, 0-145 rads, four dose levels).

The overall data from this and past experiments indicate that the postimplantation dominant lethal mutation rate rises linearly for both radiation qualities, but a detailed look at the lowest neutron dose levels shows the provocative nonlinear component (Figure 5.1, right). The increased response at 1 and 2.5 rads is about 2-fold above a simple linear fit but neither increase over linear expectation was significant. This nonlinearity is also evident in gamma ray data at 22.5 and 45 rads (data not shown). Complementary findings are also seen in the translocation data, in which the low doses of neutrons (up to 10 rads) appear to show an excess response.

The low dose data on abnormal sperm morphology (Figure 5.2) show interesting differences in response related to time of measurement. The pooled data for weeks 5 and 6 (35 and 42 days postexposure, respectively) display a generally good linear fit, and significant effects at all doses except 1 rad. However, this linear curve is composed of two extremely nonlinear components with the greater low dose effect at 5 weeks, and high dose effect at 6 weeks.

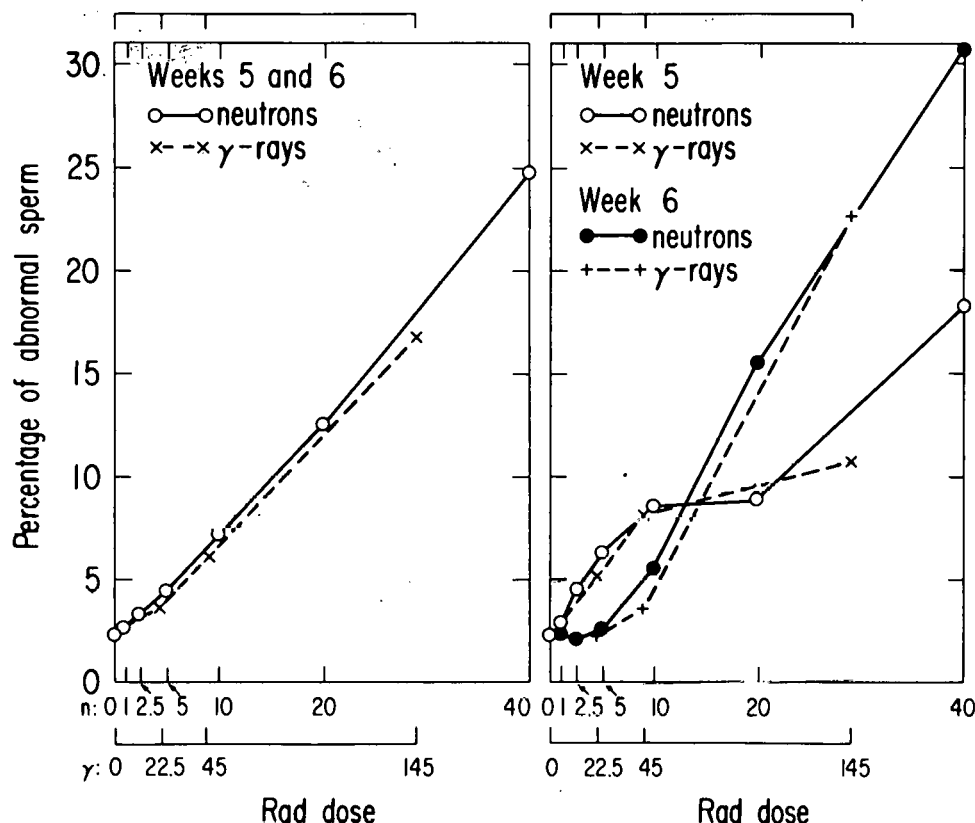


Fig. 5.2. Percentage of abnormal sperm sampled from preparations made from both epididymides. The left panel pools the data from the fifth and sixth weeks (measured on the 35th and 42nd days postexposure) and the right panel presents the two weeks separately. Estimates are based upon 1800 to 3600 sperm per sampling period (600/male).

Interpretation of the lag in response at the higher doses is difficult because the cell stage at risk is not entirely clear.

Finally, the data for dominant lethal mutations were studied on a dose by week basis with the hope that the low dose excess response might be attributed to a specific germ cell maturation stage interacting with dose. However, at the 1, 2.5, and 5 rad doses, the difference between irradiated and control was apparently distributed randomly across all 5 weeks, although statistically significant differences did occur for weeks 1, 3, and 5 (mature sperm, early spermatids, and primary spermatocytes). The possibility exists that there is significantly less germ cell killing at these extremely low doses, thus permitting more induced and spontaneous lethals or lethal equivalents to become involved in conception and development to the blastocyst stage.

GENETIC EFFECTS OF AMERICIUM-241

D. Grahn, B. H. Frystak, J. J. Russell,¹ C. H. Lee, and
A. Lindenbaum²

Americium may have a slightly different metabolic behavior than the other transuranic elements (ICRP Publication 19, 1972). To determine whether this difference is correlated with different genetic response in the testis, we initiated a preliminary test using a single injected dose of 10 $\mu\text{Ci/kg}$ of ^{241}Am given intravenously to 100-day-old B6CF₁ mice. The level of gonadal retention was only 50% to 60% of that seen for ^{239}Pu . Retention through the first 100 days after injection remained unchanged, as for plutonium, and preliminary autoradiography indicates that the microdistribution is similar to that seen for plutonium. The estimated whole gonad dose for the measured burden of about 350 picocuries per gram of gonadal tissue was 0.092 rad/day, slightly above the dose delivered by the ^{239}Pu dose of 5 $\mu\text{Ci/kg}$. The genetic responses were below those expected. Frequencies of abnormal sperm between 50 and 100 days after injection were 2.56% for the control and 2.69% for irradiated mice, compared to a value of about 4.3% expected on the basis of the plutonium data. The observed mutation rate during a 10-week dominant lethal series performed between 125 and 195 days after injection was 0.0097 lethals/gamete compared to the 0.0165 anticipated. In fact, the dominant lethal response to americium was not statistically significant ($P = 0.3$). Translocation frequency at 60 days was no more than about 0.2%, when 0.3% would be expected, although this difference is clearly not significant in a sample of only 1000 metaphase plates. Testis weight loss may be as expected, or even slightly in excess of expectation, but again sampling was limited. The remaining mice in this initial study were sacrificed at 230 days and the results of the various end points are conflicting. The frequency of abnormal sperm was now significantly higher in the americium group than in the controls (5.01% vs. 3.09%), while the frequency of translocations had fallen to zero in a sample of 1000 scored metaphases.

Autoradiographic studies are still incomplete, and the possibility that americium might be more mobile than plutonium in the interstitial tissues will be further evaluated.

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6. THERAPY OF POISONING BY RADIOACTIVE AND NONRADIOACTIVE METALS

INTRODUCTION

Maryka H. Bhattacharyya

The overall objective of this program is the development of methods for decorporation of toxic metals encountered as a result of developing energy technologies. Part of the research effort is directed toward the transuranic actinide plutonium because of its high radiotoxicity and the fact that plutonium, in many respects, is prototypical of other actinides and polyvalent heavy metals. In addition, we are investigating other toxic heavy metals of concern in energy programs. Present work includes: (1) investigation of the absorption, deposition, retention, and excretion of actinide compounds and the influence of alterations in their physicochemical properties on these end points; (2) metabolic and therapeutic studies with a nonradioactive toxic metal, lead, with particular emphasis on its retention and decorporation in the young; (3) selection and evaluation of potentially useful agents for therapeutic decorporation of toxic metals based on information derived from the above research. For economy and statistical validity, the mouse is the principal species for whole body studies. For extrapolation of results toward man, other species such as the dog are also being used.

Significant advances in our research program over the past year are described in the following sections. To summarize some of the highlights from recently obtained results:

1) We have completed a set of carefully planned experiments with beagles designed to aid in the development of an optimal therapy protocol with diethylenetriaminepentaacetic acid (DTPA) for decorporation of plutonium (and probably americium) from man. It was found that (a) use of five daily injections at 0.18 mmole/kg instead of 0.036 mmole/kg, the clinically accepted dose for man, initiated soon after exposure to plutonium, significantly improved removal of plutonium; and (b) following these first five doses, daily treatment was not significantly better than twice weekly treatment and it appears that intramuscular administration can replace intravenous administration.

2) A model for simulating wound exposure to plutonium has been established in the mouse. This model will enable us to carry out therapeutic removal studies using one of the two exposure modes relevant to the occupationally exposed population (the other mode being inhalation).

3) A collaborative study with D. Grahn (reported in Section 5) of the genetic consequences of high LET irradiation of gonads was extended to include ^{241}Am .

4) In a collaborative study with R. P. Larsen (Radiological and Environmental Research Division), the gastrointestinal absorption of Pu(IV) in mice measured at the maximum permissible concentration (MPC) for plutonium in drinking water was found to be (a) the same as that for Pu(IV) and (b) 50 to 100 times the value used by the International Commission for Radiological Protection to set the MPC for plutonium in drinking water. These results bear the important implication that the MPC for plutonium in drinking water may need to be lowered.

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¹Now in Environmental Physiology and Biomedical Effects of Energy Transmission Groups.

DEVELOPMENT OF AN OPTIMAL DTPA THERAPY PROTOCOL FOR DECORPORATION OF ACTINIDES

A. Lindenbaum, E. S. Moretti, and J. J. Russell

Studies were continued to provide data needed for the development of an optimal protocol for DTPA treatment of human workers accidentally exposed to actinide radioelements such as plutonium. Beagles previously injected intravenously with monomeric ^{239}Pu -citrate were treated with DTPA according to various regimens for 12 weeks in order to determine the effects of (1) initiating therapy using five daily intravenous DTPA injections at 0.18 mmole/kg (2) administering DTPA daily (6 days/week) vs. twice weekly, (3) starting treatment at 1 hour vs. 6 hours, and (4) using an intramuscular rather than intravenous mode of DTPA administration. Initiating daily treatment at a dose of 0.18 mmole/kg for 5 days before switching to 0.036 mmole/kg was clearly superior to the daily regimen using 0.036 mmole/kg (the currently accepted clinical dose) throughout. Skeletal plutonium levels were reduced to 32% of pretreatment levels using the higher dose at the start of therapy compared to 48% for the lower dose regimen. In addition, daily treatment with DTPA was somewhat more effective than twice weekly treatment, as was treatment starting at 1 hour rather than 6 hours. Daily injections via the intramuscular route (0.036 mmole/kg) appeared to be somewhat better in the removal of plutonium from bone than were daily intravenous injections (these two routes were initiated following five daily intravenous treatments with 0.18 mmole/kg and were continued to 12 weeks). The most effective regimen tested (five daily DTPA treatments at 0.18 mmole/kg starting at 1 hour, followed by daily intramuscular DTPA injections at 0.036 mmole/kg) achieved a fourfold reduction in skeletal plutonium levels compared to the level achieved using a standard regimen of twice weekly intravenous treatments at 0.036 mmole/kg starting at 6 hours.

WOUND EXPOSURE TO PLUTONIUM IN THE MOUSE

M. H. Bhattacharyya and D. P. Peterson

A model for simulating wound exposure to plutonium was established in the mouse utilizing an intramuscular injection into the gastrocnemius of ^{239}Pu in 2 N HNO_3 . Analysis of the kinetics of plutonium translocation from the wound indicated that (1) approximately 5% of the injected dose deposited in the skeleton and 3.2% in the liver by 24 hours, and (2) translocation of plutonium from the injection site was quite reproducible. The standard error of the mean for both skeletal and liver plutonium levels was about 8% of the mean at 24 hours. Analysis of the response to intravenous DTPA administration indicated that translocation of plutonium from the wound site to liver and skeleton continued even after five daily intravenous injections of DTPA. Measurements of the fecal excretion of plutonium following intravenous DTPA administration thus did not give a good measure of skeletal plutonium levels in the case of wound exposure. In order for our skeletal:fecal plutonium ratio method (Bhattacharyya, M. H., et al., Health Phys. 34, 549, 1978) to be useful for the estimation of skeletal plutonium levels, the source of translocating plutonium must be removed prior to or at the start of chelation therapy.

TESTICULAR PLUTONIUM AND AMERICIUM: RETENTION AND THERAPEUTIC REMOVAL

J. J. Russell, A. Lindenbaum, and D. Grahn¹

In order to evaluate further the potential genetic risk of testicular retention of alpha-emitting radionuclides, and the effect of removal therapy with DTPA, an experiment was initiated with ^{241}Am -citrate to parallel the ^{239}Pu -citrate work to date. The genetic data are summarized in Section 5 of this Report. Male B6CF₁ mice received 10 $\mu\text{Ci/kg}$ of ^{241}Am -citrate via intravenous injection. Treated animals were administered DTPA (0.25 mmole/kg) intraperitoneally starting 6 days after actinide injection and continuing twice weekly for a total of 12 weeks. Preliminary findings, through 100 days, of radiochemical analyses for americium and plutonium include: (1) a nearly constant testicular burden of 0.025% and 0.050% of the injected dose, respectively; (2) a testis weight loss of 8% and 18%, respectively; (3) an average testis dose rate of 0.09 and 0.17 rad/day, respectively; and (4) a 4- to 5-fold reduction of testicular burden by DTPA therapy for both actinides. Autoradiographic and cytogenetic studies on the testes are in progress.

GASTROINTESTINAL ABSORPTION OF PLUTONIUM

M. H. Bhattacharyya, A. Lindenbaum, R. P. Larsen,² R. D. Oldham,² and E. S. Moretti

In studies on the gastrointestinal absorption of plutonium and the effect of oxidation state, plutonium solutions were administered by gavage or ad libitum to mice either in 0.01 M bicarbonate, a concentration very similar to that in drinking water, or in 0.1 M citrate. The plutonium concentration ($^{237}\text{Pu} + ^{239}\text{Pu}$) was the same as the maximum permissible concentration (MPC) in drinking water (5 pci/ml, 80 pg/ml). Results obtained are in several ways contrary to those of other investigators: (1) there was no significant difference between the gastrointestinal absorption of Pu(VI), the form that would be present in drinking water, and that of Pu(IV), the form that was used almost exclusively in earlier experimental programs; (2) citrate did not markedly enhance the gastrointestinal absorption of plutonium; (3) the absorption factor measured for Pu(IV) (2×10^{-3}) was about two orders of magnitude higher than the gastrointestinal absorption factor used to set the MPC for soluble plutonium in drinking water (3×10^{-5}), indicating that the MPC for plutonium in drinking water may need to be revised.

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METABOLISM AND DECORPORATION OF LEAD IN THE YOUNG RODENT

E. M. Sorensen, E. S. Moretti, E. Saltz,¹ M. H. Bhattacharyya, and
A. Lindenbaum

Groups of young hybrid mice were administered a single intravenous injection of lead citrate containing 10 mg Pb/kg labeled with 15 μ Ci of ^{210}Pb /kg. This was followed, three days later, by the first of fifteen 0.25 mM/kg intraperitoneal treatments, as follows: the calcium forms of ethylenediaminetetraacetate (EDTA), diethylenetriaminepentaacetate (DTPA), dibutyl ester of DTPA (Bu_2DTPA), dihexyl ester of DTPA (Hex_2DTPA), Bu_2DTPA and DTPA, Hex_2DTPA and DTPA, or an equal volume of saline (0.15 ml). Treatment was repeated once daily for 5 days, followed by 2 days of rest, for 3 weeks. Five days after the last treatment (26 days after the lead citrate injection) the mice were sacrificed for comparison of lead distribution within various tissues, organs, and the entire carcass, as measured by the 0.047 MeV gamma emission from ^{210}Pb . Treatment of mice with either EDTA or DTPA alone was the most effective in reducing the level of lead in the brain; 49% and 43% reductions, respectively, were produced. The skeletal deposits, as measured in the femurs and closely reflected in the whole body values in all groups, were mobilized to the greatest extent by DTPA alone and combined DTPA and Hex_2DTPA . These treatments reduced lead levels in the femurs and whole body by 31% to 33% of levels in control mice. The observed correspondence in lead decorporation between the femur and carcass suggests that most of the lead removed from the body was mobilized from skeletal deposits. In tissues other than brain and bone, most of the lead was lost without chemotherapy. Lead levels in kidneys, liver, spleen, and lung were reduced by 89 to 97% of pretreatment levels by Day 26 in saline-treated controls; the additional effect of therapy on lead removal from these organs was small. Chelation therapy thus had the greatest effect on lead levels in the two important organs, brain and skeleton, where the lead burden was stable with time. In addition, although esterification of DTPA did not enhance lead removal over that seen with DTPA alone, treatment with DTPA alone was somewhat more effective than EDTA alone for removal of lead from skeleton, blood, kidneys, liver, and spleen.

Preliminary experiments with neonatal rats (14 days of age) indicated that more lead was deposited in the brains of young rats than adults (0.23% of the injected dose vs. 0.03%, respectively). In addition, it was observed that, with time, lead left the brains of both groups of animals without treatment (25-30% reduction in 2 weeks).

DTPA-INDUCED EXCRETION OF PLUTONIUM INTO DOG BILE

M. H. Bhattacharyya and D. P. Peterson

Using bile duct-cannulated dogs, a study was completed on the measurement of DTPA-induced excretion of monomeric plutonium ($^{239}\text{Pu(IV)}$ citrate) into the

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bile. A single, intravenous DTPA injection (0.18 mmole/kg) increased the concentration of plutonium in bile approximately 20-fold. The DTPA-induced excretion of plutonium into bile, however, could account for only about 40% of the plutonium lost from the liver following DTPA administration. These data contrast to our results obtained earlier in the rat, where biliary excretion of plutonium following DTPA administration accounted well for the DTPA-induced loss of plutonium from the liver. Thus, there appears to be a species difference in the mechanism of DTPA removal of plutonium from the liver of rat and dog. The striking increase in biliary excretion of plutonium in the dog following DTPA treatment implies, however, that fecal excretion of plutonium following DTPA treatment can be used as a measure of tissue-deposited plutonium levels using the S/F method (Bhattacharyya, et al., *ibid*) independent of whether man is more like rat or like dog in liver retention characteristics.

7. TOXICOLOGICAL EVALUATIONS OF PROCESSES FOR UTILIZATION OF COAL

INTRODUCTION

William P. Norris

The Divisional program to identify and evaluate toxicological and environmental problems associated with combustion and/or conversion of coal has gained momentum during the past year. Two emerging technologies for coal utilization--fluidized-bed combustion (FBC) and high BTU coal gasification--are under active investigation. A proposal to begin the toxicological evaluation of a third technology, the magnetohydrodynamic process (MHD), was prepared and submitted to DOE. In addition, there have been limited studies of fly ash collected from the precipitators of conventional coal-burning electrical generating stations.

To date, the primary sources of materials for these toxicological studies have been the two 6-inch diameter, experimental fluidized bed combustors (one pressurized at 3-10 atmospheres, one at atmospheric pressure) operated by the Argonne National Laboratory Division of Chemical Engineering, and the HYGAS pilot plant for high BTU coal gasification operated by the Institute of Gas Technology for the Department of Energy. Corresponding studies of MHD will begin with a process development unit operated by the Argonne National Laboratory Engineering Division. The cooperation of engineering staff associated with these facilities is essential to the collection of samples and proper extrapolation of results of these biological studies to full-scale commercial systems. We gratefully acknowledge their many contributions.

More recently, we have been in contact with other laboratories having similar involvements with engineering developments and/or biological testing. The objectives are to obtain effluent samples from larger installations, some of which use different engineering designs, and to verify techniques for chemical analyses and biological assays.

Biological testing of materials collected from these processes is proceeding on two levels. Level One is represented by tests that use one of a variety of bacterial and in vitro mammalian cell systems to provide indications of toxicity and potential carcinogenicity within a few weeks. So far, the most useful of these have been the Ames Salmonella assay for mutagenicity and the V79 Chinese hamster cell assay for cytotoxicity and cellular transformations. However, a number of other potentially useful Level One assay procedures have been developed to the point of application.

Level Two testing involves the exposure of laboratory rodents. Most of these exposures, so far, have been via inhalation of respirable fly ash collected from the final filters of a fluidized bed combustor. This has required

development of exposure chambers and techniques for reaerosolization and size classification of such particulate material. A second approach is to expose rodents to the total, diluted effluent of fluidized bed combustion, and a facility for this purpose has been constructed adjacent to the atmospheric pressure combustor.

In Both Level One and Level Two testing, physical and chemical characterization of effluents and process streams is of primary importance. This is done collaboratively with Argonne's Analytical Chemistry Laboratory and Division of Energy and Environmental Systems. Data are being collected to characterize both the inorganic and organic content of fly ash from FBC and the organic composition of HYGAS process streams and quench waters. Techniques have been developed for chemical class fractionations of organics from both these processes, with the result that the most toxicologically significant of the fractions have been tentatively identified in Level One testing.

The most significant accomplishments of the program during the past year result from the steadily increasing collaboration of staff in biological, chemical, and engineering disciplines. These interactions have produced a better comprehension and interest in the solution of the problems to be faced in bringing emerging technologies to commercial status. More specifically, the Division now possesses a larger and improved battery of test procedures which provide a better capability for Level One assays. During this same period, facilities for pulmonary exposures of rodents have been put in place, and Level Two testing of FBC effluents has begun. The efforts of the Analytical Chemistry Laboratory in chemical class fractionation of FBC effluents and HYGAS process oils, combined with results of Level One testing, have shown that the most mutagenic components from both these sources reside with polar organic materials. Finally, a relationship between FBC operating conditions and the mutagenicity of the effluent stream is beginning to emerge. This latter subject will be explored in more detail during the coming year.

The eleven reports that follow describe results from component efforts that, together, represent our current approach to an integrated assessment of the environmental and toxicological consequences resulting from emerging technologies for utilization of coal. In addition to the work described in these reports, mention should be made of the role of the Carcinogenesis Group in the development of rapid and reliable test systems for evaluating the neoplastic effects of coal combustion effluents, with the aim of complementing other ongoing test systems in the Division. Four systems are currently being examined: (1) in vitro culture of rat trachea in the presence of effluents, for detection of carcinogenic potential in the lung by identification of morphological and biochemical changes; (2) the quantitative measurement of hydrolytic lysosomal enzymes released from rat alveolar macrophages after in vitro exposure, as an indication of toxicity in the cell defense system and of cocarcinogenic activity in the lung; (3) the use of the early appearance of histochemically identifiable cellular changes in rat livers after in vivo exposure, as a predictor of subsequent tumor formation; and (4) biochemical or immunological detection of cellular chromosomal changes indicative of or correlated with neoplastic alterations. These four systems are described in Section 14, Carcinogenesis.

All four systems show potential usefulness, and testing with fluidized bed combustion effluents is under way with the first two models. Present data are still preliminary but suggest that samples from the effluents should be valuable for the verification and validation of each of the test systems.

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CHARACTERIZATION AND FRACTIONATION OF COMPLEX ORGANIC MIXTURES FROM FLUIDIZED BED COMBUSTION AND GASIFICATION OF COAL

S. Bourne,¹ A. Jirka,¹ and P. T. Cunningham¹

The Analytical Chemistry Laboratory supports the Divisional coal toxicology program by analyzing and preparing samples for biological testing. The preparation includes extraction of organic material from the original matrix (fly ash, coal dust, water, filters, etc.), fractionation of the organic material into chemically distinct cuts, with chemical characterization of each cut. The objectives of the work include (1) complete extraction of gram quantities of organics from the matrix; (2) quantitative separation of the complex mixtures of organic compounds into distinct chemical classes, with minimal chemical modification of the compounds; and (3) chemical characterization of the mixtures.

To date, the Analytical Chemistry Laboratory has prepared cuts for biological testing from HYGAS pretreater and quench waters and tars, HYGAS recirculation oils and char/oil slurries, fly ashes from both atmospheric and pressurized fluidized bed combustors, and filters from the combustor effluent lines. Extraction techniques have included sonic agitation or simple shaking followed by centrifuging, elution from columns, Soxhlet and separatory funnel extractions, and sublimation from particle surfaces. Separation techniques have included liquid-liquid and solid-liquid partitioning, distillation, and adsorption and gel permeation chromatography. All fractions have been fingerprinted by gas chromatography (GC) and infrared (IR) analysis.

The HYGAS samples show a wide range of polarity, starting with the nonpolar straight-chain hydrocarbons and aromatic hydrocarbons. The more polar cuts, especially from the quench waters, contain a predominance of phenols. The very polar cuts, which are gummy and insoluble in normal IR solvents, give poor IR spectra showing smeared-out hydroxyl, carbonyl, and heteroatom bands. The fly ash fractions show minor amounts of hydrocarbon material; most of the material is polar and elutes from the gas chromatograph with difficulty. The oily material extracted from filters in the combustor effluent line appears to be mainly polynuclear aromatics.

Our continuing efforts involve development of a new, chemically very gentle technique of polarity separation from Teflon beads, sublimation of materials from fly ash surfaces, and development of sophisticated GC/mass spectrometry and GC/IR systems for chemical characterization.

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CHEMICAL AND PHYSICAL CHARACTERIZATION OF PARTICULATE EFFLUENTS FROM THE ATMOSPHERIC FLUIDIZED BED COMBUSTION OF COAL

F. R. Kirchner, E. T. Kucera,¹ W. P. Norris, J. O. Hutchens,²
P. T. Cunningham,¹ S. S. Danyluk, P. C. Brennan, and W. T. Kickels

Physical and chemical characterization of particulates derived from the fluidized bed combustion of coal was performed on material ("raw" fly ash) obtained from the final (woven wire mesh) filter of the bench scale atmospheric pressure fluidized bed combustor (AFBC) in the Chemical Engineering Division and on samples after reaerosolization with liquid nitrogen (see following report). Scanning electron microscopy showed that the fly ash has a very irregular, spongelike surface conformation, which was independent of particle size and the reaerosolization process. The surface area of the ash, measured by nitrogen adsorption, was $13 \text{ m}^2 \text{ g}^{-1}$ for the raw material received from the final filter and $15 \text{ m}^2 \text{ g}^{-1}$ for the reaerosolized materials, in which the particles were $< 50 \text{ } \mu\text{m}$. Auger electron spectroscopy of the surface of the fly ash indicated that there were no significant differences following reaerosolization in the relative concentrations of C, Cl, Ca, and S in the surface layers.

As a first step in the chemical characterization of the final filter material, a sample of fly ash both before and after reaerosolization with liquid nitrogen was analyzed by spark source emission spectroscopy (Table 7.1). Twenty-two elements were observed, with only one element (beryllium) showing an increase in concentration after reaerosolization. Seven of the elements were appreciably soluble in water (Table 7.1). In addition, mercury was measured by atomic absorption and found to be present at $5.29 \pm 0.26 \text{ ppm}$ in both raw and processed ash.

Particle counts made on reaerosolized fly ash with electrical mobility and optical size analyzers indicated that a large proportion of particles were in the respirable size range ($< 5.0 \text{ } \mu\text{m}$).

A SYSTEM FOR REAEROSOLIZING FLUIDIZED BED COMBUSTOR FLY ASH FOR ANIMAL EXPOSURES

F. R. Kirchner, J. O. Hutchens,² W. P. Norris, P. C. Brennan, and
W. T. Kickels

The purpose of this work was to develop a system for exposing small laboratory animals to reaerosolized fly ash under controlled conditions. A new technique using liquid nitrogen as a carrier for the purpose of reaerosolizing fly ash derived from the final filter of a fluidized bed combustor of coal has been developed. Initial attempts at reaerosolization were made by nebulizing an aqueous suspension of fly ash. However, because emission spectroscopy of

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Table 7.1. Elemental Concentrations of "CT4" AFBC Fly Ash before and after Reaerosolization and in a 24-Hour Aqueous Extract of Fly Ash, Determined by Spark Source Emission Spectroscopy^a

Element	Raw Fly Ash	Reaerosolized Fly Ash	Water Extract of 10 gm Raw Fly Ash (ppm/100 ml H ₂ O)
Al	>10%	>10%	400
B	350	350	-
Ba	600	600	390
Be	30	50	-
Ca	3%	3%	25,000
Co	10	10	-
Cn	1,000	1,000	-
Cu	30	30	-
Fe	10%	10%	-
K	1.7%	1.7%	-
Li	60	60	8
Mg	500	500	1,500
Mn	300	300	8
Mo	20	20	8
Na	1.5%	1.5%	760
Ni	300	300	-
Pb	35	35	2
Rb	100	100	-
Si	>10%	>10%	4
Sr	1,000	1,000	500
Ti	8,000	8,000	-
V	75	75	100

^aValues listed are ppm unless otherwise indicated.

the filtered aqueous suspension medium indicated that the water had dissolved some of the fly ash, liquid nitrogen (a very poor solvent under these conditions) was chosen to replace the water. Particulates in the respirable size range ($< 5.0 \mu\text{m}$) tend to form aggregates that require substantial energy for disaggregation. The aggregates were broken up by sonication in liquid nitrogen at the triple point. The suspension was dispersed from a liquid nitrogen Dewar under pressure while being maintained in suspension with a mechanical stirrer. The stream of liquid nitrogen bearing the suspended particulates was directed to a cylindrical 4-inch diameter Plexiglas evaporation chamber, where the nitrogen was mixed with dry, filtered air from four radially opposed air inlets. The liquid nitrogen rapidly evaporated, leaving the particulates suspended in the air stream. The air stream was warmed to room temperature and directed to an animal exposure chamber. The mass loading of particulate in the exposure chamber was 0.017 mg/litre.

Physical and chemical characterization of the reaerosolized fly ash (see preceding report) indicated that the process did not alter the elemental composition, the surface area, or surface chemistry. Additionally, the biological activity as measured by H. E. Kubitschek using the Ames Salmonella test was not altered.

To date, only final filter fly ash material from the atmospheric pressure fluidized bed combustor in the Chemical Engineering Division has been used for reaerosolization. However, the technique may be applicable to other particulates, a possibility that is currently under investigation.

THE FLUIDIZED BED COMBUSTION TOTAL EFFLUENT TOXICOLOGY SYSTEM

R. Kumar,¹ J. O. Hutchens,² W. P. Norris, F. R. Kirchner, and
P. T. Cunningham³

The objective of the total effluent toxicology system of the atmospheric pressure fluidized bed combustor is to provide a facility for the direct exposure of biological test systems to the entire effluent from a fluidized bed combustor (FBC), rather than to only portions of the effluent, such as the fly ash. The system is composed of a delivery system and an exposure chamber. It provides for the dilution of the FBC effluent with filtered and conditioned air, aging of the effluent-air mixture in an illuminated atmospheric effects simulator (AES), and exposure of mice and other biological test systems to this mixture under controlled environmental conditions in a biological test chamber.

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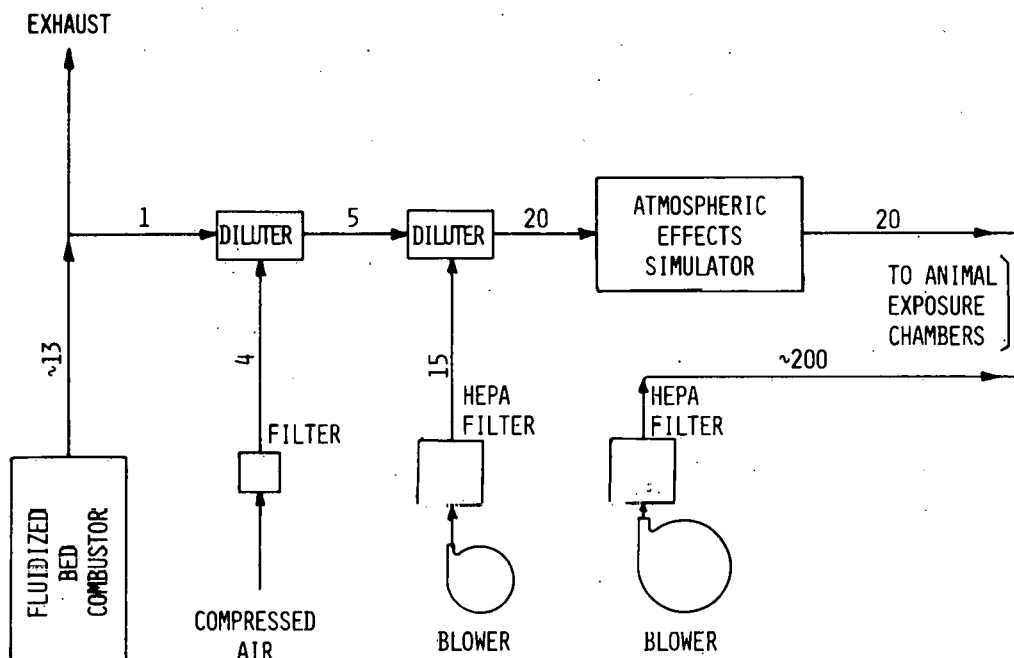


Fig. 7.1. FBC-effluent delivery system. The flow rates shown are in nominal cfm.

A schematic diagram of the delivery system is shown in Figure 7.1. The effluent source is an atmospheric pressure, 6-inch diameter, experimental fluidized bed combustor in the Chemical Engineering Division. The gas cleanup system on this combustor consists of a primary cyclone, a secondary cyclone, and a final filter. Approximately 1 standard cubic foot per minute (scfm) of the gaseous effluent from either before or after the final filter is diluted with 4 scfm of filtered, dry air. Following a second dilution with 15 scfm of filtered air from a dedicated blower, the effluent enters the AES. The AES has Pyrex glass panels in the front and back, and is illuminated with a bank of Vita-Lite fluorescent lights. The AES provides for a gas residence time of about 13 minutes. The aged effluent-air mixture from the AES, as well as 200 scfm of clean, conditioned air from another dedicated blower, is delivered to the biological test chamber.

The biological test chamber is a room about 10 x 4 x 8 feet made of sheet aluminum. Six animal exposure chambers, each about 30 x 30 x 8 inches high,

are mounted in two vertical stacks of three chambers each. The test mice are in gang cages placed inside these exposure chambers. Each of these exposure chambers can be fed with adjustable proportions of the effluent from the AES and the clean air supply, so that in these chambers the overall FBC effluent dilution ratios may range from 20 to 1000 or higher. The biological test chamber, as well as the insides of the animal exposure chambers, is maintained at 68 to 70°F and 45% relative humidity. The animals to be used in the first series of experiments are male B6CF₁ mice.

The detailed design of the various components of the FBC effluent toxicology system has been completed and the system will be operational during the summer of 1979.

CELLULAR ASSAY SYSTEMS

The preceding four reports dealt primarily with the characterization of the effluents under study, and with methods for exposing biological test systems to these effluent delivery systems. The following five reports focus on the Level One tests. The first three use the Ames Salmonella assay; the fourth compares the response to the Ames test with growth inhibition in mammalian cells; the fifth uses two additional mammalian cell systems; and the sixth reports one approach to the development of a new enzymatic test.

MUTAGENICITY OF COAL FLY ASH RECOVERED FROM ELECTRIC POWER PLANT PRECIPITATORS

H. E. Kubitschek and L. Venta¹

A major solid effluent produced by coal combustion for electric power generation is coal fly ash. In 1974 U.S. power plants burned an estimated total of 600 million tons of coal, producing some 50 million tons of fly ash, of which about 5% was released to the atmosphere. Using the Ames Salmonella reversion assay, Chrisp et al. measured the mutagenicity of the respirable fraction of fly ash collected from the smokestack breeching of a large power plant burning low sulfur, high ash coal (Chrisp, C. E., et al., Science **199**, 73, 1978). Their dose response curve for fly ash mutagenicity in strain TA1538 gave approximately 30 revertants per plate per mg fly ash extracted with serum. They did not report measurements of mutagenicity of the great bulk of the ash, about 95%, trapped in precipitators and hoppers.

¹ Summer 1978 participant in the Undergraduate Honors Research Participation Program, Northwestern University.

We obtained six samples of fly ash from precipitators and hoppers of several midwestern electric power plants, all burning low sulfur, high ash coal, and measured mutagenicity with two *Salmonella* strains, TA98 and TA1538, in the Ames *Salmonella* system, with and without the microsomal activation system. Both serum and dimethyl sulfoxide were used as extractants, and serum extractions were carried out for as long as 4 weeks at 37°C. Both benzo(a)pyrene and a dimethyl sulfoxide extract of fly ash from a fluidized bed combustor were used as positive controls. No mutagenicity (< 0.3 revertants/mg) was observed for any of the samples in some 30 tests involving the two strains and different conditions of extraction. (For example, see Figure 7.2.) To the extent that our samples are representative of fly ash from other U.S. electrical power plants, our results indicate that the great bulk of the fly ash from coal-fired generating plants is far less mutagenic than the ash actually discharged from the stacks.

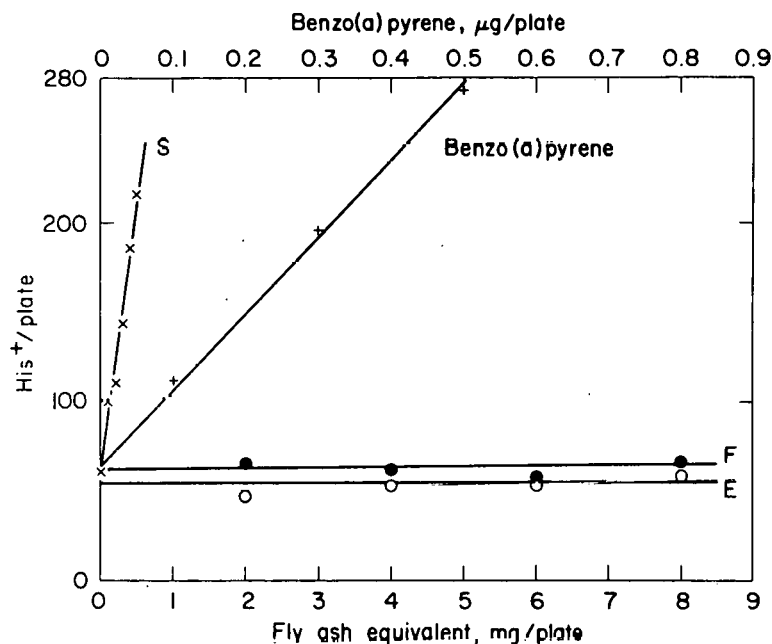


Fig. 7.2. Fly ash mutagenicity in the Ames *Salmonella* system with Strain TA98. Mutation responses (His⁺ revertants) for precipitator fly ash samples, E and F, are compared with those for two reference samples, benzo(a)pyrene and a standard mutagenic fly ash sample, S, from a fluidized bed combustor. Benzo(a)pyrene was metabolically activated (25 µg rat liver microsome, fraction S-9, per ml); the other samples were not. Fly ash samples were extracted with dimethyl sulfoxide.

MUTAGENICITY AND CHEMICAL CHARACTERIZATION OF EFFLUENTS FROM FLUIDIZED BED COMBUSTORS

H. E. Kubitschek, D. A. Haugen, S. Bourne,¹ D. M. Williams, and D. Venters

Ames Salmonella assays were performed on particulate effluents from the two experimental bench scale fluidized bed combustors (FBC's) operated by the Chemical Engineering Division--an atmospheric (AFBC) and a pressurized (PFBC) combustor, both of which burned bituminous, high sulfur (5.5%) coal. For each, fly ash was collected on a porous metal filter downstream from the primary and secondary cyclones, at a temperature of about 70°C for the AFBC and about 170°C for the PFBC. Our values for the mutagenicity of fly ash from these FBC's are shown in Table 7.2, where they are compared with those from another bench scale FBC, and with values reported for fly ash from the

Table 7.2. Mutagenic Activities of Coal Combustion Effluents and Urban Air Particulates in Strain TA98

	Source	Revertants per μg	Organic Carbon (%)	Revertants/ μg of Organic Carbon
Fly ash	PFBC ^a	0.34	2.7	12
	AFBC ^a	1.2	9.7	12
	Off-site AFBC ^a	1.9	-	-
	Electric power ^b plant stack	0.03	6-12	0.2 - 0.5
Urban airborne particulates	Buffalo ^c	3.5	85	4
	Berkeley ^c	4	85	5

^aExtracted with dimethyl sulfoxide.

^bResults of C. E. Chrisp et al. (Science 199, 73, 1978), extractions with serum.

^cFrom results of R. Talcott and E. Wei (J. Nat. Cancer Inst. 58, 449, 1977), assuming organic material was approximately 85% carbon. The particulates from Buffalo required metabolic activation.

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breeching of an electric generating plant, and also with some values reported for urban airborne particulates. All of these values were obtained with the same bacterial strain, *Salmonella* TA98. The specific mutagenicity of each of the FBC ashes were an order of magnitude larger than that observed for the electric power plant; but the FBC activities were less than that observed for urban airborne particulates in Buffalo and Berkeley. When values of specific mutagenicity are based on organic carbon content, both the fly ash from the FBC and the urban air particulates had values of about 1 to 2% of those for the carcinogen benzo(a)pyrene (300 to 400 revertants per μg).

In order to characterize further the organic components, fly ash was extracted with phosphate-buffered saline to yield an insoluble residue that was further extracted with a series of organic solvents. The extract was applied to a silica gel column and six fractions of increasing polarity were eluted. These fractions contained 90% of the activity applied to the column. Almost all of the mutagenic activity was distributed in the final four fractions (Table 7.3), representative of 4-6 ring aromatics, phenols and chemicals containing carbonyl and nitrile moieties, alcohols, and carboxylic acids and other strongly polar compounds; the activities of the first two low polarity chromatographic fractions and of the saline extract were negligible. The most polar fraction (eluted with methanol) contained the greatest mass and mutagenic activity. Fraction 4, however, had the greatest specific activity, and

Table 7.3. Mutagenicity of PFBC Particulate Effluent Fractions

Fraction	Chemical ^a Constituents	mg/Fraction	Mutants/ μg	Mutants/Fraction $\times 10^{-3}$
1	Long chain aliphatics; minor unsaturated compounds.	17.3	0	0
2	30% aliphatic, 70% light aromatics (2-3 rings).	2.8	0	0
3	Heavy aromatics (4-6 rings) 5 major components.	9.9	61	604
4	Mixture of phenols, carbonyls, nitriles.	3.4	250	850
5	Phenols, carbonyls, alcohols.	36.6	18	660
6	Carboxylic acids and other very polar compounds.	276.2	4	1104

^aThe six fractions were obtained by elution of silica gel with hexane, hexane plus 5% benzene, hexane plus 50% benzene, chloroform, ethyl acetate, and methanol.

was composed primarily of a mixture of phenols and of chemicals containing carbonyl and nitrile moieties. The specific mutagenic activity of this fraction was very high, approximately 50% that of benzo(a)pyrene; this fraction must therefore be considered potentially very hazardous.

Thus, our results with fly ash fractions clearly indicate the presence of many different kinds of mutagenic compounds. A broad spectrum of mutagenic compounds also has been observed for process stream mutagens produced in a high BTU gasification process and may well be representative of most kinds of coal combustion and conversion technologies.

A high-volume air sampler with a glass fiber filter was installed on an exhaust duct into which the PFBC effluent was vented downstream from the final fly ash filter. The temperature at this filter was 40-50°C. Under typical operating conditions, 0.5 to 1 gram of effluent was collected on this filter, as a mixture of variable proportions of black particulate material and a condensed dense yellow-orange oil. The oil-like material is not believed to contain elemental sulfur, is insoluble in water, but is readily soluble in organic solvents such as hexane or dichloromethane. Gas chromatographic and mass spectrographic analysis revealed the presence of more than 30 aromatic compounds, and infrared analysis revealed the presence of carbonyl moieties.

In order to evaluate the biological activity of the condensate, filters containing either primarily the yellow oil or a mixture of oil and particulates were extracted with dichloromethane, the solvent was evaporated, and the residue was dissolved in dimethyl sulfoxide and evaluated by the Ames Salmonella assay. The condensate extracts from both the yellow oil and the more mixed sample were directly mutagenic and effective at 2-5 µg/ml or 20 revertants per µg, indicating the presence of potent mutagens. The particulate effluents collected at higher temperatures from the porous metal filter were also directly mutagenic, supporting the interpretation that the particulate effluent collected on the glass fiber filters represents effluent that passed the primary porous metal filter. In addition, the results suggest that the yellow condensate contains volatile mutagens that were not removed by deposition on ash collected by the porous metal filter and, therefore, that this condensate preferentially contains the most volatile of the mutagenic vapors produced during combustion. Thus these condensates, which contain high concentrations of organic materials, probably are the best source of material for initial identification and characterization of potent organic mutagens produced by fluidized bed combustion.

MUTAGENICITY OF OIL PROCESS STREAMS IN THE HYGAS COAL GASIFICATION PLANT

B. S. Hass, S. S. Dornfeld, S. Bourne,¹ and D. M. Smith²

Three different samples from oil process streams of the HYGAS plant have been examined for mutagenicity using the Ames *Salmonella* assay, with strain TA98 activated with S-9 rat microsomal enzymes. Two samples were from the oil recirculation loop--one collected when the plant was not in operation (Sample A) and the other collected during a test run (Sample B)--and the third sample was from the char-oil slurry stream.

Sample A and the char-oil slurry were fractionated on silica gel in exactly the same way. Both showed considerable specific mutagenicity (revertants/ μ g) in Fractions 5 and 6 which are composed of polar compounds (Table 7.4). Fraction 3 of Sample A, containing 3-5 ring aromatics, showed an unusually high specific mutagenicity, which might be related to the presence of

Table 7.4. Mutagenic Response of *Salmonella typhimurium* Strain TA98 to Oil Recirculation Loop Sample A, the Char-Oil Slurry Sample, and Their Fractions

Sample	Silica Gel Elutant	Chemical Classes Identified	Oil Recirculation Loop Sample A			Char-Oil Slurry		
			Revertants/ μ g	Total Sample (g)	Revertants/Sample ($\times 10^{-6}$)	Revertants/ μ g	Total Sample (g)	Revertants/Sample ($\times 10^{-6}$)
Raw			0.30	1000	300	36.01	475	171
Total nonvolatile extract			1.90	12.9	24.5	5.32	10.5	55.7
Fraction 1	Hexane	Long chain aliphatics	0	2.9	0	0	1.6	0
Fraction 2	5% hexane in hexane	30% aliphatic 70% aromatic	0	3.5	0	0	2.8	0
Fraction 3	50% benzene in hexane	3-5 ring aromatics	35.72	1.3	46.6	2.40	0.6	1.4
Fraction 4	Chloroform	Phenols, carbonyls, nitriles	0	0.9	0	0	1.0	0
Fraction 5	Ethyl acetate	Phenols, alcohols, carbonyls	4.20	3.3	13.9	13.30	3.1	41.2
Fraction 6	Methanol	Carboxylic acids and other very polar compounds	14.10	0.51	7.3	14.12	0.2	2.7

¹Analytical Chemistry Laboratory of the Chemical Engineering Division.

²Sponsored by the Argonne Affirmative Action Program.

certain mutagenic chemical species that could accumulate with the changing combustion conditions of the shut-down process of the previous run, since lower temperatures are known to facilitate the synthesis of polycyclic aromatic hydrocarbons. The mutagenic balance (mutagenicity of a whole sample compared to that of the sum of its components) was excellent in the nonvolatile extract of the char-oil slurry (92%, corrected for loss of mass in the fractions) and for Sample A, excluding Fraction 3, it was 87%.

Sample B of the oil recirculation loop was assayed as collected ("raw") and as divided into volatile (b.p. < 200°C) and nonvolatile portions (Table 7.5). Chemical recovery was 98% and the mutant recovery in the two portions was 109% of the mutagenicity of the undivided sample. Thus, if the low specific mutagenicities of the raw and volatile samples are statistically valid, it is possible to obtain reasonable mutant recoveries between the sample as collected and its components.

The fractionation procedure for the nonvolatile portion of Sample B was modified from that used for Sample A. Since polar material tended to adhere to the silica gel column, the very polar materials were first removed on a Teflon column before separation on the silica gel column. A comparison of the mutagenicities of the various fractions in Sample A (Table 7.4) with those in Sample B (Table 7.6) indicates that the mutagenic response was altered by the removal of the very polar fractions from Sample B. For example, mutagenicity appeared in the chloroform fraction which had earlier contained none.

One conclusion from the testing of the HYGAS oil streams is that the non-volatile portions seem to produce lower mutagenic responses (revertants/μg) in the Ames *Salmonella* assay than organic samples from process and product streams of coal liquefaction conversion technologies as reported by Epler, J. L., et al. (Mutat. Res. 57, 265, 1978).

Table 7.5. Mutagenic Response of *Salmonella typhimurium* Strain TA98 to Oil Recirculation Loop Sample B

	Revertants/μg	Total Sample (g)	Revertants/sample (x 10 ⁻⁶)
Raw sample	0.13	417.1	54.2
Volatile component	0.13	404.0	52.5
Nonvolatile component	1.90	3.5	6.7

Table 7.6. Mutagenic Response of *Salmonella typhimurium* Strain TA98 to Fractions of the Nonvolatile Portions of Sample B

Sample	Column ^a	Eluant	Revertants/ μ g	Total Sample (g)	Revertants/ Sample ($\times 10^{-6}$)
3	T	Methanol-water (2:1)	1.40	4.60	6.4
5	SG	Hexane	0.24	3.23	0.6
5	SG	5% benzene in hexane	5.43	0.08	0.4
7	SG	50% benzene in hexane	2.60	0.39	1.0
8	SG	Chloroform	9.61	0.43	4.1
9	SF	Ethyl acetate	1.24	0.27	0.3
10	SG	Methanol	1.22	0.05	0.06
11	SG	Water	1.49	0.01	0.02

^aSG = silica gel; T = Teflon.

COMPARISON OF MUTAGENICITY AND GROWTH INHIBITION OF WATER QUENCH PROCESS STREAMS FROM THE HYGAS GASIFICATION PLANT

B. Hass, S. S. Dornfeld, and T. Matsushita

A water quench process stream has been sampled and analyzed for mutagenic responses in the Ames *Salmonella* assay, using metabolically activated strain TA98, and for growth inhibition in mouse myeloma cells. This sample was collected from the gasifier pretreater quench stream. A second sample from the oil recirculation loop quench process stream, which collects recoverable oil evolved by the gasifier, was also analyzed for mutagenicity.

The growth inhibition tests used mouse myeloma cells (MPC 11; 66.2 sub-clone) grown in liquid suspension of Dulbecco's modified Eagle's medium containing 20% heat-inactivated horse serum. Culture growth was determined by a Coulter counter attached to a multichannel analyzer. This instrument enables the counting of viable cells according to size and distribution characteristics which are calibrated and correlated by vital staining techniques. Growth curves are plotted, and then the ED₅₀ (effective dose to reduce the growth rate by 50%) is determined graphically.

No bacterial mutagenicity was found in the pretreater quench stream sample, as collected, or in its aqueous component, and these were not examined

for growth inhibition. The water of the quench stream contained a tarlike, insoluble material which was extracted repeatedly according to the scheme shown in Figure 7.3. Fractions T-3 through T-7 (see figure) were compared for mutagenicity and growth inhibition in mouse myeloma cells (Table 7.7). There is a poor correlation between mutagenicity and growth inhibition in Samples T-4, T-5, and T-6. In addition, the sample with the largest total mutagenicity value, T-7, displayed a very low value for growth inhibition. The data also indicate that the process of extraction altered the response of the components. For example, Fraction T-7, one of three fractions derived from T-4, displayed a higher total mutagenicity than the parent T-4.

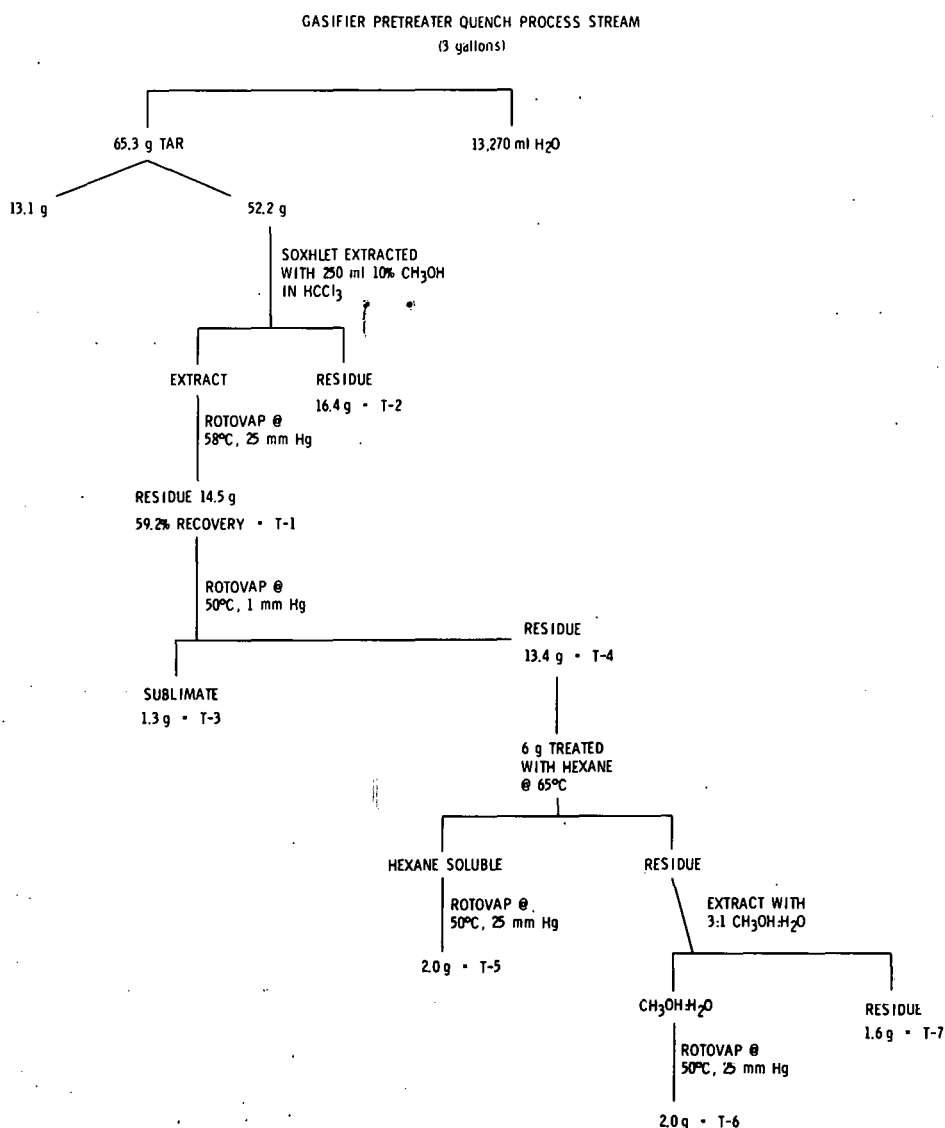


Fig. 7.3. Chemical separation scheme for a sample from the gasifier pretreater quench process stream. Chemical separation performed by S. Bourne and A. Jirca of the Analytical Chemistry Laboratory (see report by S. Bourne et al. in this Section).

Table 7.7. Mutagenicity and Growth Inhibition of Extracts from Gasifier Pretreatment Quench Stream Samples from the HYGAS Coal Gasification Plant

Fraction	Fraction Identification	Total Sample (g)	Mutagenicity		Growth Inhibition (ED ₅₀ , µg/ml)
			Specific (revertants/µg)	Total (revertants/sample × 10 ⁻⁶)	
T-3	Soxhlet sublimate of extract	1.3	0	0	>280
T-4	Parent ^a	13.4	0.24	3.22	112
T-5	Nonpolar	2.0	0.57	1.14	75
T-6	Polar	2.0	0.30	0.06	62
T-7	Very polar	1.6	2.6	4.16	120

^aT-3, T-5, and T-7 are derived from T-4.

The oil recirculation quench stream sample was fractionated on a silica gel column and eluted with solvents of increasing polarity. The significant mutagenic responses were from polar samples. The final four polar fractions produced the specific mutagenicities of 0.24, 1.32, 6.59, and 1.03 revertants/µg, respectively.

In conclusion, the comparison of the bacterial mutagenicity and growth inhibition in mammalian cells indicates that the two are poorly correlated, and suggests that a comprehensive evaluation of potential health hazards requires the use of more than one end point.

CYTOTOXIC EFFECTS OF THE EFFLUENTS FROM FLUIDIZED BED COAL COMBUSTION

G. R. Lankas, M. M. Elkind, and E. M. Buess

The effects of the effluents produced by the bench scale pressurized fluidized bed coal combustor have been studied in mammalian cells in culture. Small particulates up to about 10 µm in diameter (fly ash), organic extracts of the fly ash particulates, and condensate material deposited on glass fiber filters located at the terminal end of the effluent stream were tested in V79 Chinese hamster cells for cytotoxicity of the effluents alone and in combination with sunlight-simulating light, and for mutation induction at the 6-thioguanine-resistance locus. In addition, studies on the potential for induction of cell transformation in C3H/10T1/2 mouse cells, indicative of oncogenic activity, have been initiated using the effluent stream condensate material.

Fly ash was incubated at 37°C in cell culture medium containing 15% fetal calf serum at a concentration of 2 mg/ml. At various times the media were filtered and incubated directly with V79 cells for one week to measure colony forming ability. For periods up to 5 days of extraction, the resulting

medium did not affect cell survival, but extraction for longer periods significantly reduced cell survival (maximum effect, 1% survival produced after 12 days of extraction). This 12-day extract did not photosensitize the cells to Westinghouse Sun Lamps (FS 20, ~ 290 – 340 nm with a maximum intensity at ~ 313 nm). However, when this same fly ash was extracted with a series of organic solvents, and the soluble material was eluted on a silica gel column with solvents of increasing polarity, it was found that the two most polar extracts, ethyl acetate and methanol, significantly enhanced the cell killing produced by the sunlight-simulating light.

The organic extract of the condensate was toxic to the cells exposed for 24 hours to concentrations over $10 \mu\text{g/ml}$. Interaction studies with the sunlight-simulating source indicated that pretreatment of the cells with the condensate material significantly increased the cell killing over that caused by the light alone. This effect was shown to be dependent upon both the length of pretreatment with the condensate and its concentration. Maximum photosensitization occurred when the cells were pretreated with $50 \mu\text{g/ml}$ of the condensate for 3 hours at 37°C followed immediately by the radiation (Figure 7.4). In addition, pretreatment of the cells with the condensate as above, followed by irradiation with General Electric Black Lights (F40 BL, emission maximum 365 nm) over a dose range that is not cytotoxic, also results in significant photolethality.

To date, experiments on induction of mutations at the 6-thioguanine-resistance locus of V79 cells with the condensate material have yielded negative results. Additional studies are in progress employing a cell-mediated activation system to determine whether this material requires metabolic activation for detection of mutagenic activity.

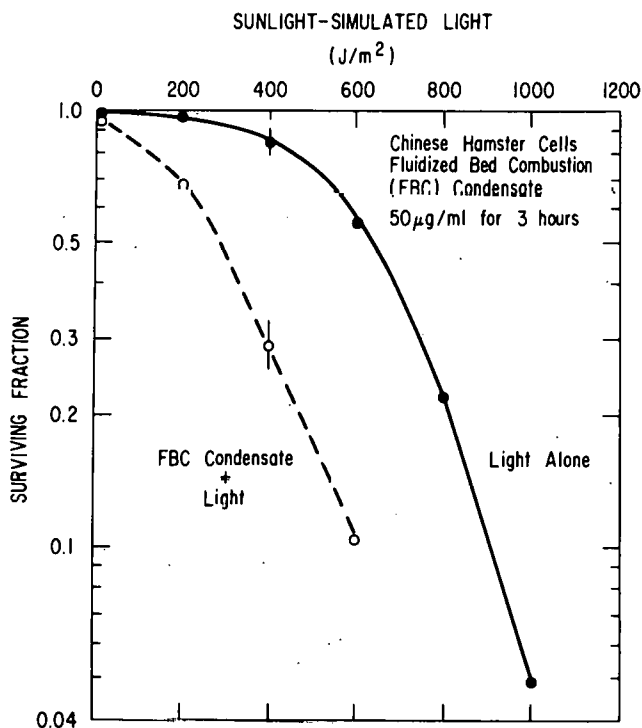


Fig. 7.4. Effect of fluidized bed combustion condensate on the sensitivity of Chinese hamster cells exposed to sunlight simulating light (~ 290 – 340 nm). The cells were treated with the condensate for 3 hours at a concentration of $50 \mu\text{g/ml}$ just prior to the near-UV irradiation. Growth medium containing 0.5% acetone was used as the carrier solution. Uncertainties are standard errors.

MAMMALIAN TOXICOLOGY

The final two reports deal with the exposure of mice to effluents from fluidized bed combustion and with the development of a new enzymatic test to detect mammalian tissue response to in vivo exposure of the animal to energy-related chemical pollutants.

TOXICOLOGICAL RESPONSES OF MICE EXPOSED TO REAEROSOLIZED FLY ASH FROM ATMOSPHERIC PRESSURE FLUIDIZED BED COMBUSTION

P. C. Brennan, W. P. Norris, F. R. Kirchner, T. M. Seed,¹
S. P. Stearner,² D. M. Buchholz,¹ W. T. Kickels, and S. S. Dornfeld

Male B6CF₁ mice were exposed to reaerosolized fly ash in three separate experiments using the exposure system described by Kirchner et al. earlier in this Section. The first two were of short duration, 30 hours (6 hours/day for 5 days), and were designed primarily to test the logistics of the exposure system, although preliminary evaluations of these mice were made. The fly ash used in these experiments, designated "Fly Ash A," was a composite of material from start-up and steady-state operation. The third experiment consisted of exposure of mice for 100 hours (~ 6 hours/day, 5 days a week) over a 4-week period, and was designed to determine the organs and tissues at risk from fly ash in the respirable size range. The fly ash used was designated "CT4," and was obtained during steady-state operation only. As in Fly Ash A, the particles were predominantly < 5 μ m. We learned during the course of the 100-hour exposure that Fly Ash A is ~ 10 times more mutagenic in the Ames Salmonella test than is CT4 fly ash.

Because it was not known which tissues or systems would be primarily affected by fly ash, a number of biological responses were examined. However, special attention was given to lung alveolar macrophage function because we knew from preliminary studies that alveolar macrophages readily engulf fly ash particles. Mice exposed to Fly Ash A for one week showed a marked proliferation of alveolar macrophages and these macrophages were full of engulfed fly ash when examined by light microscopy. However, macrophages in culture from exposed mice were unable to kill a challenge dose of Staphylococcus aureus as effectively as those from control mice. This finding suggests an impaired function in the first line of defense against inhaled pathogens following short-term exposure to fly ash.

As with alveolar macrophages, other cell renewing systems showed an early proliferative response following short-term exposure to fly ash. Bone marrow stem cells, intestinal crypt cells, and the proliferative response of spleen lymphocytes were all increased following the short exposure to Fly Ash A. On the other hand, measurements of pulmonary compliance and histopathological studies did not show any appreciable change.

¹Cellular Indicators Group.

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Only an early evaluation has been made on mice exposed to the less mutagenic CT4 fly ash for 100 hours. No effect was observed on any of the end points described above. Late effects of this exposure will be determined at 3-month intervals in the remaining mice.

BIPHENYL AS A MODEL SUBSTRATE IN THE CHARACTERIZATION OF CYTOCHROME P-450-DEPENDENT MONOOXYGENASES

D. A. Haugen, K. M. Suhrbier, W. C. Bauman,¹ and J. R. Milstead²

Biphenyl is under evaluation as a model substrate for characterizing cytochrome P-450-dependent monooxygenase systems because its metabolism is distinctly altered by in vivo exposure to polycyclic aromatic hydrocarbons, because several stable metabolites may be detected and quantitated, and because its metabolism is representative of that of polycyclic aromatic hydrocarbons. The relative and absolute amounts of three primary metabolites, 2-, 3-, and 4-hydroxybiphenyl, formed in the presence of different forms of partially purified rat liver microsomal cytochrome P-450, have been used to distinguish the substrate specificity of these different forms. The same approach has been used to detect alterations in the in vitro metabolism of biphenyl by liver microsomes isolated from rats treated with either phenobarbital or 3-methylcholanthrene. The former causes up to a 10-fold increase in the formation of 3-hydroxybiphenyl, and the latter causes up to a 30-fold increase in the formation of both 2-, and 3-hydroxybiphenyl, each having only a minor effect on the formation of 4-hydroxybiphenyl. Based on these observations, a procedure is under development whereby the metabolism of biphenyl is used to detect qualitative and quantitative changes in the monooxygenase systems of selected tissues in response to in vivo exposure to minimal levels of environmental chemicals, including products of coal combustion.

¹Fall 1978 participant in the Undergraduate Honors Research Participation Program, Nebraska Wesleyan University.

²Summer 1978 participant in the Undergraduate Honors Research Participation Program, Southern Illinois University.

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8. HUMAN HEALTH EFFECTS AND RISK ASSESSMENTS

INTRODUCTION

Douglas Grahm

This group's research is concerned with four general areas relevant to the quantification of the possible adverse health effects of energy technologies. These are as follows:

1) Analyses of existing data concerning the health effects of energy technologies to determine what adverse effects have occurred and to produce better estimates of what adverse effects might be expected from expansion of the same or similar technologies.

2) Construction of data bases and exploratory data analyses for the purpose of determining the degree to which the health impact of energy technologies may be modified by the socioeconomic composition of the exposed populations.

3) Development of age structured population projection models which will predict the health effects of a particular energy technology scenario with greater precision than conventional single coefficient models.

4) Development of new statistical methodologies and reevaluation of standard procedures used in the estimation of the health effects of energy technologies.

The ultimate goal of these diverse lines of inquiry is to provide persons responsible for the assessment of health impacts with a unified body of fact and theory upon which they may base their analyses.

The major accomplishments of the past year include the following. Monte Carlo simulation routines for the basic DEMPAC model were developed. These routines can generate empirical error bounds for all DEMPAC projection estimates. Preliminary analyses of the Tri-state leukemia data were completed that indicate that low levels of diagnostic X-ray exposures may not be associated with an increased risk of adult nonlymphatic leukemias, as suggested by some earlier studies. Examination of the possible health consequences of the conversion by major users to coal from oil and natural gas resulting from the Powerplant and Industrial Fuel Use Act of 1978 identified a region of five states in the middle South as the most likely to experience the greatest relative impact.

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PREDICTIVE MODELS FOR HEALTH IMPACTS FROM ENERGY RELATED ENVIRONMENTAL POLLUTION: THE DEMPAC MODEL

R. T. Lundy and M. E. Ginevan

This project is designed to generate a set of computer models which will, given any initial population, estimate the number of fatalities or illnesses that may result from exposure to a given health hazard. These models represent an advance over previous procedures in that they consider the effects of competing risks, time-varying base-line conditions, and age structured populations.

The basic model was defined and implemented earlier. During this year, several modifications and extensions have been incorporated.

Originally, the DEMPAC model produced an estimate of the population structure at any future time, and estimates of the births and deaths (allocated by age and cause) that occurred during the interval between the present and the specified time. In many circumstances, however, detailed analysis of the death rate structure is of greater interest than the actual numbers. Consequently, an option allowing the generation of multiple decrement life tables based on the projected rates was added.

Histories of individual age cohorts are also often of interest (e.g., persons less than 5 years of age in 1980). Because the original DEMPAC model lumped birth cohorts in its projections, a modification was made that generates summary projections of selected birth cohorts in isolation.

In other cases, only a "risk estimator" is desired. This is a linear coefficient giving the relationship between a total person-dose and the resulting number of deaths or illnesses. This is strongly dependent on the population's dose history and its evolving age distribution. Thus, such a risk estimator must be specific to the initial population structure. It is valid only while the magnitude of the anticipated effects is small relative to the competing risks. DEMPAC has been modified to generate such estimators with each projection. The deviations from linearity in the estimator can be checked by recomputing it for several dose levels.

Efforts were also initiated to specify confidence intervals for the projections. This work demonstrated that for analyses of single cohorts an approximation based on the Poisson distribution provides adequate error bounds for the projected numbers of deaths. When dealing with cross-sectional projections spanning a number of incomplete cohort histories, the situation is much more complex. Therefore a set of Monte Carlo simulation routines for DEMPAC have been developed. These programs can generate stochastic realizations of the population projection for any particular pollution scenario and population structure. By executing them a large number of times (100-500) for a given set of initial conditions, empirical error bounds for all DEMPAC projection estimates may be generated.

Present efforts involve incorporation of more realistic dose response functions, construction of improved submodels for the distribution of births

by age of mother, and development of a set of routines to simulate a multiregional population with migration among the regions.

STATISTICAL QUESTIONS IN ASSESSMENT OF HUMAN HEALTH IMPACTS OF ENERGY RELATED EFFLUENTS

M. E. Ginevan

Epidemiological studies of the health effects of energy related effluents frequently arouse considerable controversy. More often than not the issues concern the appropriateness or potential biases of the statistical methodology employed. To date, my studies of these sorts of problems have involved two separate but related lines of research, real data analysis and development of new statistical methodology.

Data analyses have focused on a reevaluation of a possible link between diagnostic X-ray dosage and adult nonlymphatic leukemias. Some earlier studies have suggested that relatively small numbers of diagnostic X-ray exposures may be associated with significantly elevated risk of leukemia. Reanalysis demonstrates that the increases shown for low exposure persons (receiving less than 40 diagnostic X-rays in a 10-year period) are due to the fact that they were lumped in a category with persons who had received very high X-ray exposure (greater than 60 in a 10-year period) who showed very high leukemia risk. The significance of the finding that persons with very high X-ray exposure show very high leukemia risk is, however, problematic. Very high X-ray exposures may cause a precipitous rise in leukemia risk, but it is equally likely that undiagnosed leukemia manifests itself as a chronic disease state which results in X-ray exposures designed to determine the basis of ill health (Ginevan, M. E., Proceedings of the Second Workshop on Health Surveillance around Point Sources of Pollution, Albuquerque, NM, January 22-24, 1979, in press).

Further analyses of the relevant data will better determine the nature, if any, of the X-ray leukemia cause-effect relationship, and consider the validity of other claims (such as that X-rays cause heart disease) which have been made concerning deleterious effects of diagnostic X-rays.

Development of statistical methodology has been primarily concerned with expanding applications of the Poisson distribution to epidemiological studies, particularly those involving two population comparisons of cause of death data. This work has demonstrated that, under assumptions, the Poisson trials test using the binomial distribution is at least as good as more conventional chi-square procedures in terms of both sensitivity and power. Further, in terms of application it is both conceptually and computationally simpler (Ginevan, M. E., Health Phys., in press; Proceedings of the Biometric Society Meeting, Washington, DC, August 13-16, 1979, in press).

A more recent investigation concerns exploratory regression procedures used for hypothesis generation. In such analyses, "the best" K variable regression that can be produced from N independent variables (K much less than

N) is often judged in terms of the percentage variation explained (R^2), but the resulting R^2 of the K variable regression is an order statistic which has an expected value of much more than 0. For the best ten-variable regression, taken from 50 independent variables, the expected R^2 is ~ 0.20 for a sample size of 200. Thus, we cannot test for significance using standard procedures. Work is being initiated to develop improved procedures for assessing the goodness of exploratory regressions.

INTERACTIONS OF SOCIOECONOMIC VARIABLES AND HUMAN MORTALITY PATTERNS

D. K. Dixon-Davis, D. Grahn, J. J. Collins,¹ J. R. B. Curtiss, and R. T. Lundy

This study attempts to quantify the base-line relationship between human health, as measured by mortality, and various socioeconomic factors. In its initial phase, this study focused on 15 regions selected for their proximity to a variety of different types of energy producing facilities. Regions selected were sets of counties within 50 miles of these facilities. The selected regions sample the full spectrum of geographic, economic, and social variables in the U.S. and the full complement of current methods for generating electric power (gas, oil, coal, nuclear, and hydro).

The development of the primary data base for the 191 counties in the initial phase was completed in 1978. This data base includes mortality data for the period 1969-71 by age, sex, race, and for 28 causes as well as total mortality. Also included are data for selected socioeconomic variables derived from the U.S. Census, for air pollution (sulfur dioxide and total suspended particulates), climate (average annual maximum July and minimum January temperatures and total precipitation), for electric power production by type (nuclear, hydroelectric, fossil), and per capita consumption of beer and cigarettes (at the state level).

Regression analyses for all causes of death together in the primary data base suggest that socioeconomic factors are associated with nearly half of the variation in the mortality. When individual causes of death were examined, it was found that the different causes vary in their association with socioeconomic factors in a consistent fashion: Diseases that are thought to have a major genetic component (diabetes and congenital defects), and should therefore be relatively insensitive to socioeconomic factors, were not strongly associated. On the other hand, the proportion of explained variance in cardiovascular diseases and total cancer rates, which are thought to have a strong environmental component, was in excess of 35%. In the coming year the predictive value of regression equations will be tested on a parallel data set consisting of all mainland U.S. counties.

¹Resident Student Associate, University of Illinois, Urbana.

A major review (Curtiss, J. R. B., and D. Grahn, submitted for publication, 1979) was completed on the socioeconomic, demographic, cultural, and environmental factors influencing mortality. Fifteen variables are discussed and eight (education, occupation, industrial mix, urban residence, marital status, ethnic mix, and cigarette and alcohol consumption) are singled out as an initial set for further attention in the construction of models. The relationship of each to mortality is discussed. Unique to this review is the quantitative summary (presented as an appendix) of the variables influencing adult mortality. These numerical relationships, derived either directly or indirectly from published data, will support further modeling efforts.

COMPARATIVE EVALUATIONS OF THE HUMAN HEALTH IMPACTS OF DIVERSE ENERGY TECHNOLOGIES

C. D. Brown and J. R. B. Curtiss

The energy strategy needed to meet predicted national power demand will include a mix of technologies, each carrying its own array of potential health hazards. The impact of such hazards must be considered before implementation of these new technologies so as to ensure a healthful environment, while meeting future energy demands.

Efforts over the past year have been devoted to developing procedures that enable quantitative assessment of nonnuclear energy technology and application of these procedures to estimation of impacts from energy use scenarios. The primary emphasis of this work was on coal utilization. Other technologies considered for evaluation were the electric vehicle and the solar power satellite.

One major investigation concerned analysis of the coal fuel cycle. In this, accidental injury was considered as a major component of the overall health impact. Impact factors derived from current safety statistics, coal production, and railroad transportation statistics are shown in Table 8.1. These factors are useful estimates of the contemporary impact of coal use since they are based on mid-1970's statistics. By referencing these to specific unit requirements of a coal use cycle, it is possible to assess a coal dependent energy system in terms of its impact, through accidental injury, upon an exposed occupational and general population.

Another part of our evaluations resulted in construction of a machine readable data set containing 1975 population weighted exposures to sulfur dioxide and total suspended particulates for all counties in the contiguous 48 states. This data set has recently been used in estimation of population health impacts, and will provide a valuable resource for future efforts.

A health effects assessment for the Powerplant and Industrial Fuel Use Act of 1978 (DOE/EIS-0038D) examined possible consequences of the conversion of major users to coal from oil and natural gas. Federal Region VI (Arkansas, Mississippi, New Mexico, Oklahoma, and Texas) was predicted to experience the greatest relative impact, largely as a result of occupational and public acci-

Table 8.1. Accidental Injury Factors Associated with the Coal Use Cycle

	Fatal Injuries	Nonfatal Injuries	Unit ⁻¹
Coal mining.			
Surface	0.06	3.37	10 ⁶ T mined coal ^a
Underground	0.35	30.93	10 ⁶ T mined coal
Coal preparation	0.03	0.13	10 ⁶ T mined coal
Transportation			
Railroad worker	1.52	158	10 ⁵ loaded railcars (100 T/car)
Trespasser	2.96	1.49	10 ⁵ loaded railcars
Grade crossing	3.48	7.08	10 ⁶ train-miles (100 cars/train)

^aT = a ton of 2,000 pounds.

dental injuries related to the transportation of coal to the area. On a national basis, increased coal use as a result of the Fuel Use Act is expected to result in 41 fatal and 1300 nonfatal injuries in 1985 with 78 fatal and 2500 nonfatal injuries in 1990.

In a health assessment of electric vehicle storage batteries, three batteries for near-term use (lead/acid, nickel/zinc, nickel/iron) were extensively reviewed from the aspect of metal toxicity. As a part of this work, dose response functions for heavy metal exposure were examined. These studies demonstrated that, in the low dose range, an exponential function of the form

$$y = ae^{bx},$$

where y is the response parameter and x is the heavy metal dose, describes a number of different physiological responses and, on both practical and theoretical grounds, is preferable to other functions proposed.

Other impact assessment activities included the comparative assessment of industrial coal utilization processes (National Coal Utilization Assessment, ANL/IAPE/TM-DRAFT) and the identification of human health issues for a year 2000 scenario of a solar power satellite system.

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9. BIOMEDICAL AND ENVIRONMENTAL EFFECTS OF ELECTRICAL STORAGE SYSTEMS

INTRODUCTION

John F. Thomson

As mentioned in the 1977 Annual Report (ANL-78-90, p. 1), a new program was begun in early 1978 that was concerned with the health and environmental effects associated with the manufacture and use of storage batteries, in anticipation of increased use in utility load leveling and in electric vehicles. The program is managed jointly by the Division of Biological and Medical Research (BIM) and the Environmental Impact Statement Division (EIS). The initial emphasis was on the assessment of environmental and health effects of the complete cycle: mining and milling of raw materials, commercial production, use, recycling, and disposal of noncylable components. The project leader for the assessment phase is Dr. R. K. Sharma (EIS), and both EIS and BIM personnel have been involved in the preparation of the assessments.

The original philosophy was that once areas where additional research was needed were identified as a result of the assessment, a program would be initiated in BIM to carry out such research. However, it became apparent that there were sizeable uncertainties in the technology development, which would control future development much more than toxicological considerations would.

Therefore, the decision was made late in 1978 to formulate a research program, with the objective of obtaining data on the toxicity of metals, particularly but not exclusively those of probable significance in energy-storage systems. The metals that appear to be of importance in battery development will generally receive first attention, but the approach will be essentially generic.

The program as presently formulated consists of seven projects, most of them outgrowths or extensions of current programs, ranging from molecular and cellular studies to sophisticated measurements of performance of whole animals:

- 1) Chemical and Molecular Interactions of Metals with Biological Molecules
- 2) Effects of Metals on the Cytochrome P-450-dependent Monooxygenase
- 3) Mouse Myeloma Cells as an in Vitro Testing System for Metal Toxicity
- 4) The Effects of Metal Toxicants on Respiratory Carcinogenesis

- 5) Analysis of Pregnant and Lactating Animals as a Potential "Population at Risk" from Exposure to Toxic Metals
- 6) Effects of Battery-related Metals on Energy Metabolism, Activity, and Lifetime Performance of Mice
- 7) Dyschronogenesis and the Circadian Toxicology of Heavy Metals in the Mammal

It may be noted that most of these systems are readily amenable to the study of mixtures of two or more metals, or mixtures of metals with other pollutants, in order to study the possibilities of potentiation, additivity, antagonism, or synergism. These possibilities are important because the standards that have been established for toxic substances assume independence of action.

The major effort in this program will not begin until October 1979, and although some progress has been made in five of the seven projects during the first few months of 1979, we feel that it would be premature to report these preliminary findings in detail.

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10. BIOMEDICAL EFFECTS OF ENERGY TRANSMISSION

INTRODUCTION

Charles F. Ehret and George A. Sacher

This is a new program, on which work began in April of 1978. Its general aim is to ascertain whether or not sinusoidal electric fields of extremely low frequency (10-240 Hz) have any effect upon behavioral and neurophysiological functions, especially as indicated by perturbations of circadian oscillations in long time series, and of disturbances in ultradian episodes in the diel cycles of small rodents. This exploitation of the diagnostic significance of circadian and ultradian wave forms or "signatures" is based upon correlations between changes in signature with changes in performance, vigor, morbidity, and life expectancy (Ehret, C. F., K. R. Groh, and J. C. Meinert, Advances in Experimental Medicine and Biology, vol. 108, 185, 1978; Sacher, G. A., and P. H. Duffy, Advances in Experimental Medicine and Biology, vol. 108, 105, 1978; Sacher, G. A., and P. H. Duffy, Fed. Proc. 38, 184, 1979). The diagnostic use of circadian and ultradian signatures represents a highly sensitive, relevant, and unique approach to the problem of appraisal of potential environmental hazards.

The principal accomplishments of this year have been in the provision of controlled environment spaces for the experimental exposures, the design and construction of exposure facilities, behavioral and physiological monitoring systems, and dosimetric procedures for rats and mice (Ehret, C. F., et al., Proceedings of the 18th Annual Hanford Life Sciences Symposium on Biological Effects of Extremely-Low-Frequency Electromagnetic Fields, Richland, WA, October 16-18, 1978, in press). The initial designs described below are for 60-Hz electric fields at field strengths that can range from zero to 100 kV/m. Field strengths in the two kinds of facilities were measured with a state-of-the-art miniprobe provided by the National Bureau of Standards, with modifications developed at Argonne National Laboratory.

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DESIGN AND CONSTRUCTION OF BASIC FIELD GENERATORS FOR EXTREMELY LOW FREQUENCY ELECTRICAL FIELD STUDIES WITH SMALL RODENTS

C. F. Ehret, G. A. Sacher, A. Langsdorf,¹ and R. N. Lewis²

The basic generator consists of toroidal end plate electrodes (inner diameter = 30.5 cm) separated by lucite supporting elements at a constant distance of 30.5 cm, and is equipped with three guard rings equally spaced between the end plates (Figure 10.1). The generator may be employed to deliver horizontal fields (in the rat experiments described below) or vertical fields (in the mouse experiments described below). The end plates are of polished aluminum and their tubular periphery is of 2.5-cm diameter, like that of the guard rings. This diameter is sufficient to prevent corona discharge at applied voltages up to 30 kV and field strengths up to 100 kV/m. The available cylindrical space within the generator is just large enough to accommodate the animal cages described below; the small size of the generator permits the use of transformers of less than 100 watts to generate field strengths as high as 100 kV/m. The guard rings enable the use of small parallel plate designs in which field distortions are minimized despite the small ratio (approximately 1:1) of end plate diameter to distance between end plates. The end plates are supplied voltages from the secondary of a high voltage transformer while the guard rings are supplied from a resistive divider across the transformer secondary or from taps on the secondary.

DESIGN AND CONSTRUCTION OF CAGE FACILITIES FOR CIRCADIAN STUDIES OF EXTREMELY LOW FREQUENCY ELECTRIC FIELD EFFECTS IN THE RAT

C. F. Ehret, K. R. Groh, J. C. Meinert, and G. Svihla

The basic cage and data collection system for circadian studies in the rat is a modification of one that has been used successfully for several years in circadian studies (Ehret, C. F., et al., *Science* 188, 1212, 1975). The system consists of long-term housing for a single rat in the form of a glass cylinder, equipped with parts for ventilation, for water, and for food, and surrounded by an antenna for radiotelemetry reception of core temperature signals broadcast by a temperature transducer-transmitter implanted intraperitoneally. The radiotelemetry antenna is located within the central guard ring, which is held at ground potential in this configuration. Novel features in the electric field facility include a floor fabricated of a 2.5 x 2.5 cm mesh glass gridwork, which is suspended by nylon strands from a force transducer (Figure 10.2). Ultradian activity episodes are recorded as displacement forces each time the rat moves within the cage; transducer values recorded during quiescent phases reflect weight and weight changes. Behavioral responses are measured by means of programmed tapping and bar-press devices that are a part of each cage. In operant conditioning response paradigms, rats can

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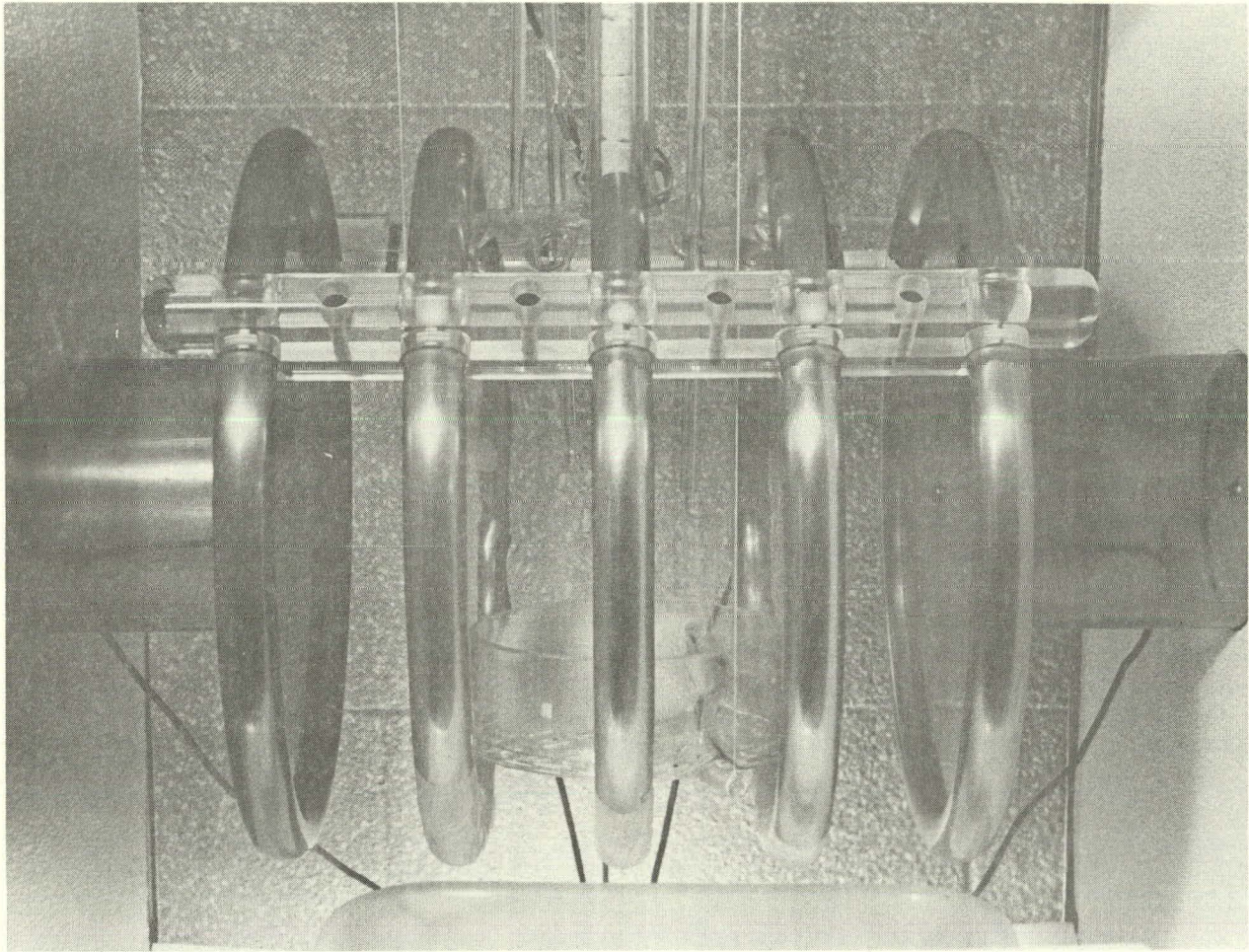


Fig. 10.1. Closeup view of horizontal field generator. The glass grid upon which the rat is seated is suspended by nylon cord. Glass food hoppers with pelleted food enter the cage top through the feeding guides. Note also the bar press (cage center right) and ball press (cage center left) devices.

be rewarded with food from motor driven automated glass food hoppers that descend from cylindrical glass hopper guides that cap the cage. In the rat cage, the E field is horizontal, and the ground plane is defined by the central guard ring that bisects the cage vertically; this places metal-free watering and feeding devices and the radiotelemetry antenna in the center of the ground plane. The cage is housed in a sound dampened booth within a controlled environment room along with nine other cages. Two such rooms are projected, and 8-track programs of the sounds of rat room activity will be broadcast constantly to the 20 animals housed in the two rooms. Booths are surrounded by aluminum foil shielding on sides and tops, but are covered by aluminum screening (7 holes/cm mesh) at front and rear to permit ventilation and the entrance of light for photo-entrainment.

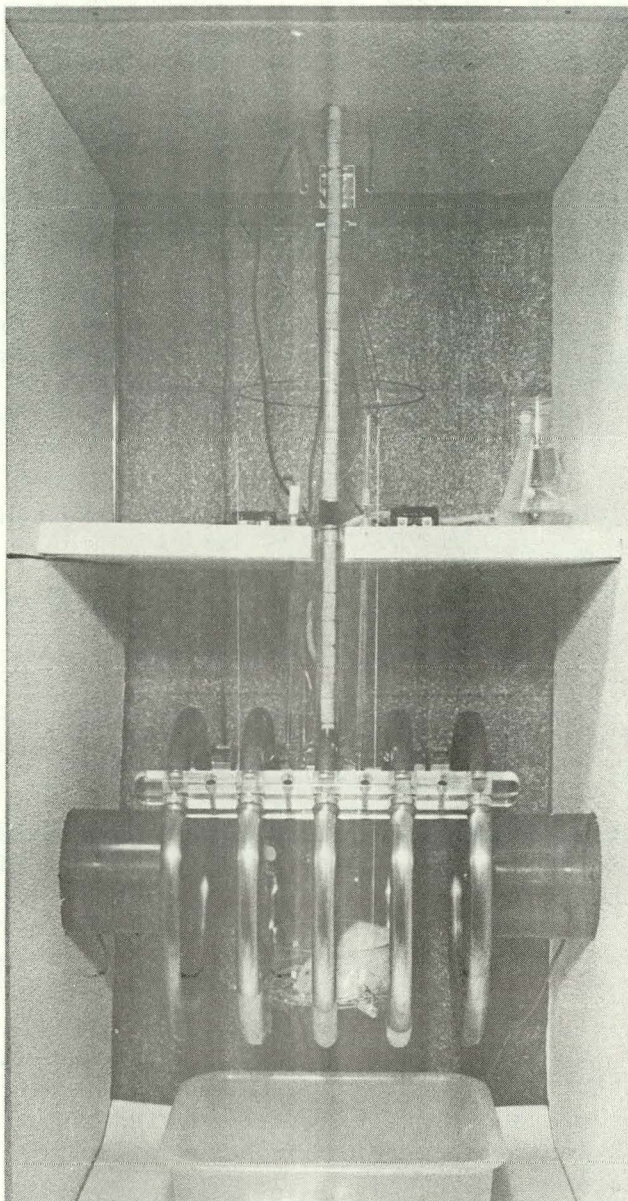


Fig. 10.2. Complete horizontal field generator unit. The lower portion shows the exposure facility consisting of the basic field generator and the caged rat within it. The rat sits on a glass grid suspended by nylon cords from a force transducer (see upper portion, ceiling). The food hoppers enter from the top of the cage. The water supply bottle is in upper right and enters the cage area as a spigot in the top cage center (cannot be seen). Limit switches in the upper portion are connected to bar-press devices for operant-controlled conditioning experiments.

An on-line controller and data acquisition system is used for programming and monitoring the various environmental factors and measured outputs. Each installation (cage) in the electric field facility produces three analog signal outputs--one for behavior, one for activity, one for temperature--which are collected automatically at variable intervals by a programmable microcomputer. Circadian variations in behavior are monitored by counting bar presses in response to audible cues given at various circadian phases, with food as a reward. Circadian activity and body weight fluctuations are recorded from impulses that originate in the force transducer described above. Signals relevant to circadian fluctuations in deep body temperature, broadcast by implanted transmitters and received by the antennae described above, are then enhanced by individual pre-amplifiers, converted from frequency to voltage, digitized, and stored as data.

EFFECTS OF HIGH-VOLTAGE FIELDS IN ENERGY METABOLISM AND BEHAVIOR OF MICE

G. A. Sacher, P. H. Duffy, and J. J. Russell

The major efforts in this project were directed toward the design and construction of cage facilities for the exposure of mice to high voltage 60-Hz electric fields, and for monitoring the diel cycles of energy metabolism, motor activity, and thermoregulation (the MAT system).

The MAT system provides automatic logging of oxygen consumption, carbon dioxide production, motor activity, and body temperature continuously on each of eight mice simultaneously for periods up to 10 days (Figure 10.3). The use of the MAT variables for defining physiological health status was established in prior publications (Sacher, G. A., and P. H. Duffy, *Fed. Proc.* 38, 184, 1979).

Cages and watering devices were constructed, utilizing materials with high dielectric constants, and minimizing the use of metals, to maintain field uniformity. The exposure and monitoring are carried out within light- and temperature-programmable environmental chambers to isolate subjects from ambient noise and vibrations.

The electric field generator employs a circular upper electrode and three guard rings to produce a uniform electric field within the metabolism module (Figure 10.4). A subsequent field mapping, utilizing the electric field probe developed by Martin Misakian of the National Bureau of Standards, showed that the field varies by no more than 3 percent within the empty cage (Figure 10.5). A high voltage patch-panel permits flexible power distribution from two 35-kV transformers to the eight individual field generators. A carefully designed interlock system prevents electrical hazards. High voltage connections are made by means of flexible high voltage cable, and cable terminations are made with a newly developed multilayer taping process. These procedures provide compact and corona-free installation.

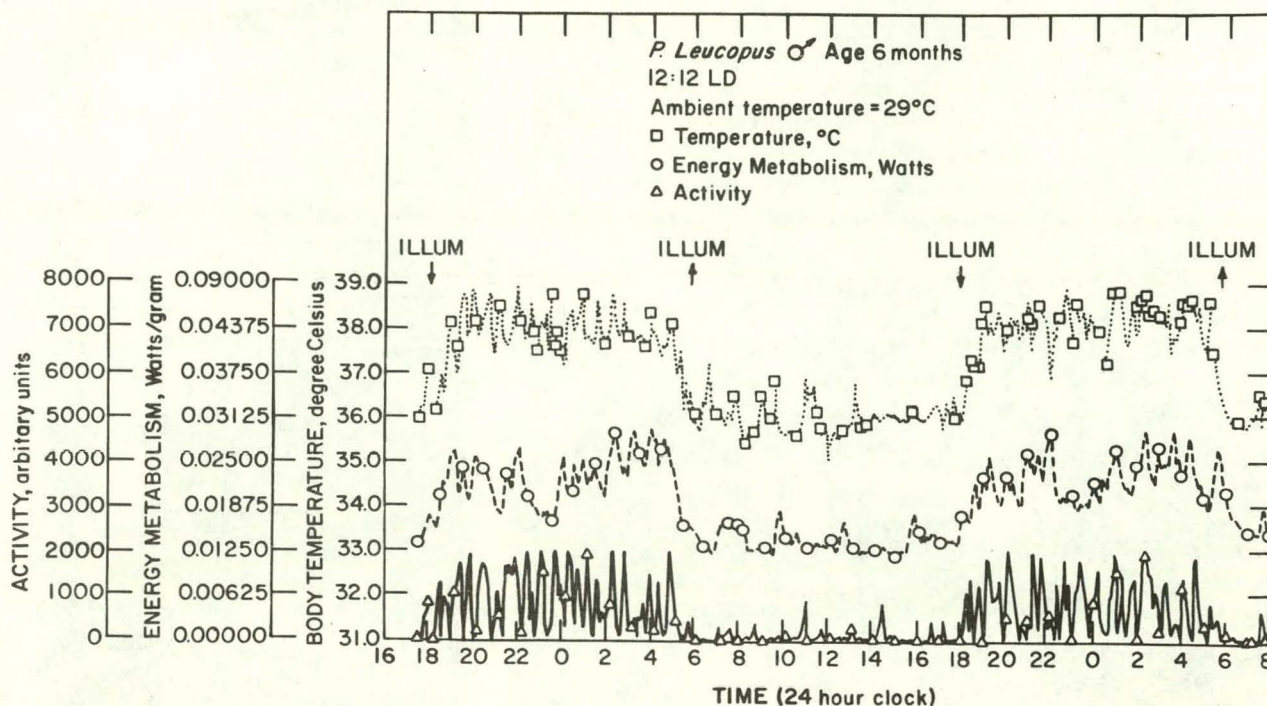


Fig. 10.3. A 40-hour sample of the energy metabolism and activity of an individual mouse. The three variables monitored are, from bottom to top: motor activity, measured as the kinetic energy of movement in the metabolism cage; rate of energy metabolism in watts per gram, estimated from rate of oxygen consumption; and deep body temperature in degrees Celsius, measured by radio-telemetry from a small temperature transducer transmitter implanted in the peritoneal cavity of the mouse. The animal subject is a young white-footed mouse, *Peromyscus leucopus*. The light-dark cycle is 12 hours of darkness (from 1800 to 0600 hours) and 12 hours of light. Ambient temperature is 29°C, which is within the thermoneutral zone for this species. Laboratory mice and wild mice give similar records under these conditions.

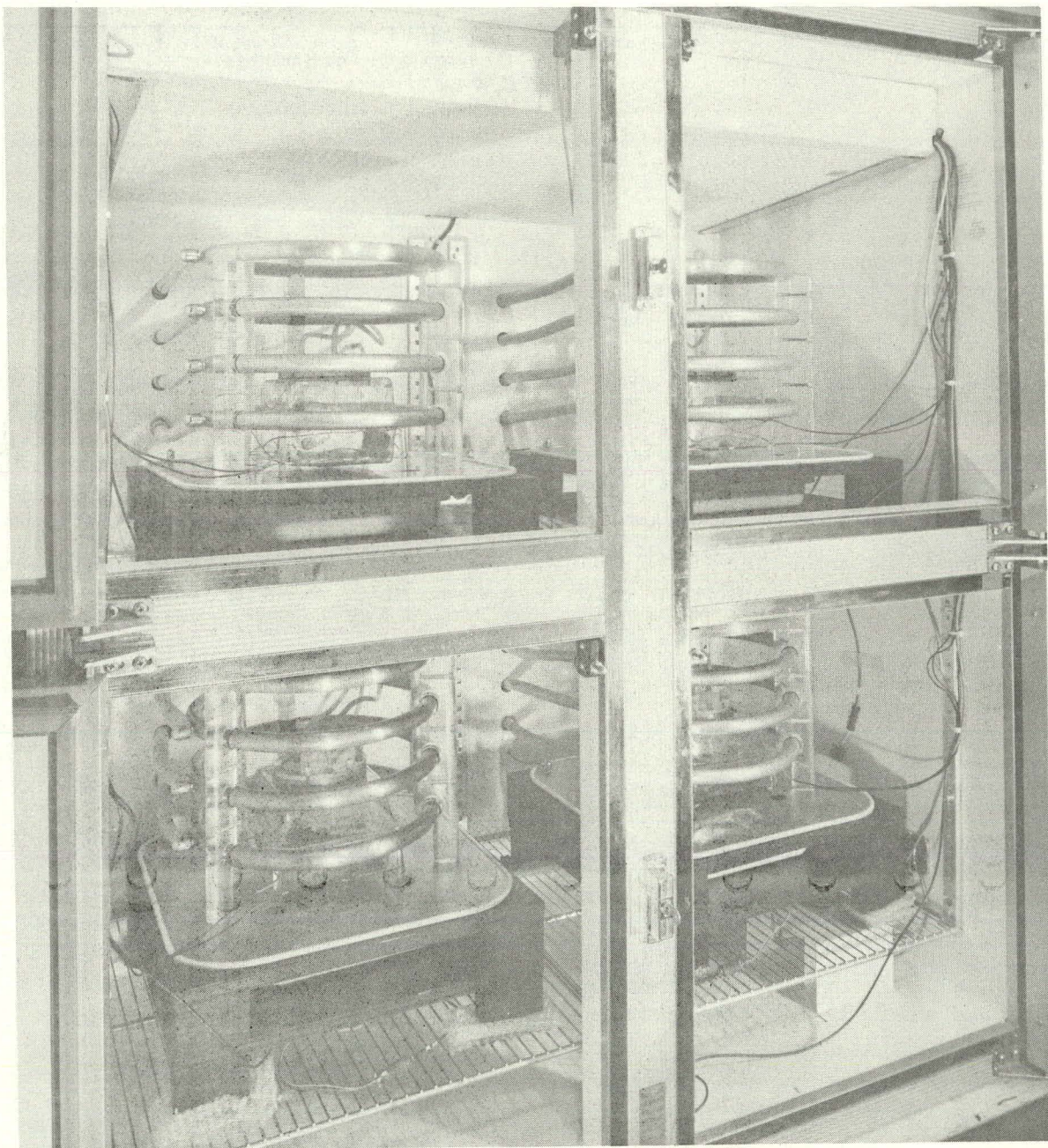


Fig. 10.4. Four individual modules for monitoring energy metabolism and activity of mice in high-voltage electric fields, shown in place within the environmental chambers. A total of eight mice can be monitored in the chamber, each equipped with independent high voltage supply and control systems. The upper electrode with 2.5-cm diameter annular edge, and the three 2.5-cm diameter guard rings are shown with high-voltage cables connected. The metabolism cage is insulated from the ground plane electrode, and is mounted on a spring and dashpot suspension below the ground electrode. The antenna for pickup of the telemetered temperature signal can be seen wrapped around the outside of the upper left metabolism cage.

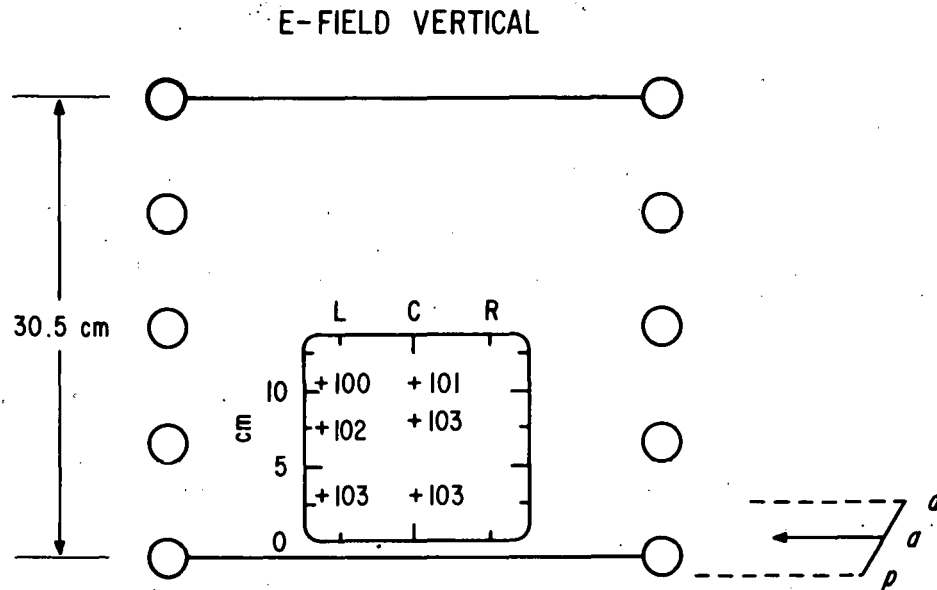


Fig. 10.5. Schematic cross-sectional view of exposure module, to show the variation of field strength within the empty metabolism cage. The field intensity in the empty field, in units of volts per meter (V/m), has about a 3% range of variation within the space that the mouse will occupy. However, this is only a confirmation of the design specifications and not an indication of the field experienced by the living animal, for the presence of the animal grossly distorts the field, and every change of position of the animal alters its local field.

A 32-channel data acquisition system controlled by an LSI-11 microcomputer has been developed for system management and data processing. Data as acquired will be stored in a magnetic tape cassette storage system. A separate microcomputer system for data plotting and simple statistical analysis incorporates a twin-disk mass storage system. The newly developed computer software emphasizes user convenience, and greatly reduces experimenter time in comparison to our previous data-management programs. More complex statistical procedures, such as time series analysis, will be carried out by the central computer in the Applied Mathematics Division.

EFFECTS OF HIGH-VOLTAGE FIELDS ON BEHAVIOR IN THE MOUSE

W. Obermeyer¹ and G. A. Sacher

Our behavioral study in mice of the effect of imposition or presence of extremely low frequency electric fields has three foci: determination of (1) perceptual and aversive thresholds; (2) changes in ongoing behavior, both in itself and as an indication of physiological change; and (3) changes in electrophysiological parameters as measures of behavioral state and brain activity.

Two Hotpack incubators have been modified to serve as controlled environment experimental enclosures. Each of these is capable of containing two experimental chambers and one is currently capable of containing two field generators around the chambers. Experimental chambers are a part of the MAT system as described in the preceding report. One of these has been modified by the addition of a metal grid which is capable of delivering a mild aversive electric shock stimulus of known intensity to the experimental animal.

In order to measure bar-press response in determining thresholds of perception and aversion, an optical switch has been designed and built which is compatible with MAT measurement and minimal field distortion. Massey-Dickinson logic modules have been assembled and programmed to permit data acquisition and experimental control for the first experimental paradigm for determination of perceptual threshold. Software parameters for eventual control of these experiments by current computer hardware have been specified. For determination of physiologic changes, a protocol has been written to use activity as a measure of the effect of electric fields on permeability of the blood:brain barrier to quaternary amines. For studies of behavioral state and brain activity, a polygraph for data acquisition and a stereotaxic instrument for electrode placement have been acquired.

¹Resident Student Associate, University of Chicago.

11. MOLECULAR BIOPHYSICS

INTRODUCTION

Steven S. Danyluk

In Molecular Biophysics, our efforts are committed to basic research in areas of molecular biology/biochemistry, biophysics, and chronobiology, coupled with supportive research for Divisional programs in toxicology and health effects of environmental pollutants generated by alternate energy technologies. Two main themes are encompassed in these studies; one deals with development of fundamental structural and biophysical information for biological systems, and the second is concerned with basic molecular and physiological mechanisms of circadian rhythms. In both instances the emphasis is on problems of major contemporary interest, namely the molecular basis of biological organization and causality.

In the structural area a range of biophysical methods, including X-ray crystallography and magnetic resonance spectroscopy, are being used to probe three-dimensional structures and properties of important classes of biological molecules. Further progress has been made in refinement of the X-ray diffraction structure for the Mcg Bence-Jones dimer. Using the interactive computer graphics system at the University of North Carolina, the coordinates were refined for nearly all of the nonhydrogen atoms of the molecule. The results have enabled a better definition of key structural features, such as the immunoglobulin fold, and have identified an anomalous region of minimal electron density arising from one of the hypervariable loops. Work is also under way on the X-ray structure determination for (Fin), a κ -type Bence-Jones protein, and preliminary results have already been obtained for the angle between variable and constant segments of the molecule. An attempt is now being made to solve the Fin structure by molecular replacement methods.

With completion of the base-line conformational analyses of di-ribonucleoside monophosphates, our attention has shifted to the impact of base modification upon conformational properties. Of particular interest here are the effects of alkylating mutagens and photohydration upon overall structure, and the consequences of the effects on function. NMR results obtained to date show that N-1 methylation of adenine rings causes a major disruption in ordered, base-stacked conformations of adenylate dimers. Implications of these findings for tRNA and mRNA structures are now under exploration. Success was achieved in synthesis of a new class of compounds in which a potent antitumor drug such as arabinocytidine (araC) is coupled via a pyrophosphate linkage to 1,2-dipalmitin. These molecular depot drugs hold promise of improved efficacy of the antitumor agent while permitting selective tissue targeting. Initial tests in the L1210 system and in the MCP-11 mouse myeloma

cell line show that araCDP-dipalmitin is indeed acting as a molecular depot or prodrug. Further testing on whole animal systems is now under way at Roswell Park.

New initiatives undertaken in the past year in the bio-structure area include the application of solid state NMR methods and development of a pulsed neutron small-angle diffractometer. Using cross-polarization and magic-angle spinning techniques originally developed for solid state studies, well-resolved high-resolution ^{13}C spectra have been obtained for biological molecules in intact tissue. These measurements, if verified by work now in progress, open up possibilities for direct structure/conformation investigations in cells and tissues under in vivo conditions. The small-angle diffractometer, scheduled for installation on the ZING-P' pulsed neutron source early in 1980, is primarily designed for exploration of operational characteristics of a pulsed diffractometer and will serve as a prototype for an instrument to be constructed at the Argonne intense pulsed spallation neutron source IPNS-1.

In the circadian studies, a particularly interesting finding relates to the effect of chronic administration of the chronobiotic phenobarbital in rats. On the basis of circadian criteria alone, it was possible to characterize a complete habituation, addiction, and withdrawal sequence. The sequence consists of (1) an early habituation effect, characterized by transient circadian dyschronism; (2) a "steady-state" tolerance or addiction effect, with remarkably stable amplitude enhancements and psi-shifts, dependent in magnitude upon phase-ratio of the zeitgeber; and (3) an abstinence syndrome characterized by severe dyschronism when the chronobiotically active drug is withdrawn.

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ALKYLATION EFFECTS ON NUCLEIC ACID CONFORMATIONS

S. S. Danyluk, C. F. Ainsworth, and M. MacCoss

Alkylation of nucleic acids by chemical mutagens and carcinogens produces a multiplicity of products depending upon the nature of the base ring, electrophilicity of the alkylating agent, and the presence of activating enzymes. For example, alkylation of adenine nucleotides can occur at six sites (Figure 11.1); strong electrophiles such as N-methyl-N-nitrosourea and N-methyl-N¹-nitro-N-nitrosoguanidine react with available oxygen donors, whereas weak electrophiles dimethyl sulfate and methyl methane sulfonate show a higher tendency to methylation at N1, N3, and N7. While the chemical modification of nucleotides by methylating agents has been studied in considerable detail (Singer, B., and J. T. Kusmierk, *Biochemistry* 15, 5052, 1976), less is known about the impact of base methylation upon nucleic acid conformation and structure. This aspect has now been explored for several base-modified diribonucleoside monophosphates.

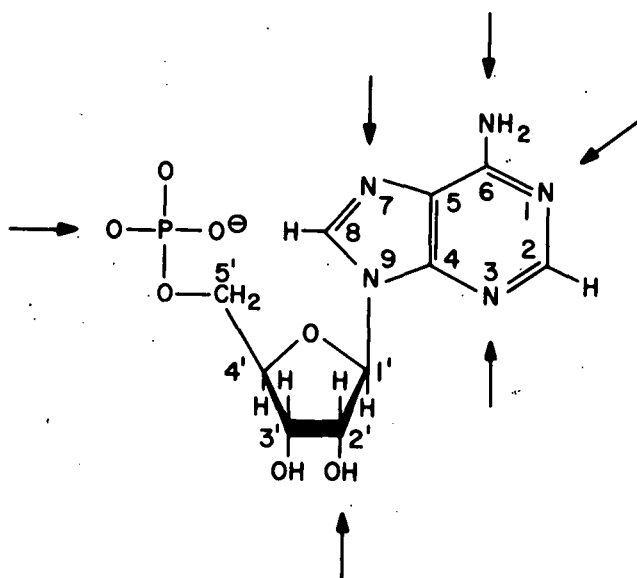


Fig. 11.1. Putative methylation sites on an adenine nucleotide.

Using the powerful alkylating mutagen dimethyl sulfate, selective methylation (Brinacombe, R. L. C., et al., *Biochemistry* 11, 2452, 1965) was achieved at the N1 adenine position of ApA, ApU, and UpA. High-resolution proton NMR spectra were recorded at 220 MHz for each of the methylated dimers ^{Me}Ap^{Me}A, ^{Me}ApU, and Up^{Me}A in D₂O under varying conditions of pD and temperature. An evaluation of conformational properties from NMR parameters followed procedures developed in earlier dimer studies (Kondo, N. S., and S. S. Danyluk, *Biochemistry* 15, 756, 1976).

Methylation of the adenine ring produces a profound alteration in dimer conformation, the change being most striking for the adenylyate dimer. Thus, covalent linkage of the methyl group at N1 with concomitant introduction of a positive charge into the purine ring causes substantial destacking of bases

in $\text{MeAp}^{\text{Me}}\text{A}$ relative to ApA, i.e., 8% versus 38%. Accompanying the destacking are shifts in ribose pucker from ${}^3\text{E}$ to ${}^2\text{E}$ and detectable decreases in gauche-gauche populations about C4'-C5' and C5'-O5' bonds. All of these effects are consistent with a less ordered conformation for the methylated dimer. Similar trends are observed for MeApU and $\text{Up}^{\text{Me}}\text{A}$, but the extent of conformational perturbation is less marked due in part to the absence of repulsive electrostatic interaction between adjacent positively charged base rings, and the existence of an initial less ordered structure for unmodified purine-pyrimidine and pyrimidine-purine dimers.

Based on these results and in view of the ubiquitous presence of methylated bases in naturally occurring polynucleotides, it is tempting to assign a role for methylated adenylate residues, and by inference N-7Me guanine bases, as conformational mediators between ordered and open structural domains of tRNA and mRNA molecules. From this perspective, methylation of available base sites by carcinogens produces irreversible conformational changes with accompanying disruption of biological function. Experiments are currently under way to test this hypothesis.

HIGH-RESOLUTION MAGNETIC RESONANCE SPECTROSCOPY OF INTACT BIOLOGICAL MATERIALS

S. S. Danyluk and H. M. Schwartz

High-resolution NMR spectroscopy of condensed state materials such as organic solids, biopolymers, and intact biological systems (cells, tissue) has been a long-standing goal of investigators in this field. Its attainment has been held back by extensive line broadening of signals for solids and ordered polymeric states (line widths of 100-1000 Hz are typical for ${}^1\text{H}$ and ${}^{13}\text{C}$ nuclei as compared with < 1 Hz widths in solutions of small organic and biological molecules), and by extremely low sensitivity, an especially critical concern for nuclei with low natural abundance such as ${}^{13}\text{C}$. These problems have been resolved to a considerable degree by recent theoretical (Pines, A., M. G. Gibby, and J. S. Waugh, *J. Chem. Phys.* **59**, 569, 1973) and instrumental developments (Schaefer, J., E. O. Stejskal, and R. Buchdahl, *Macromolecules*, **8**, 291, 1975). Using a combination of cross-polarization (CP) and magic-angle spinning techniques, dramatic reductions in ${}^{13}\text{C}$ line widths accompanied by marked enhancements in signal to noise ratio have been obtained for various polycrystalline solids and polymers. We have now extended the CP magic-angle NMR approach to measurement of high-resolution ${}^{13}\text{C}$ spectra for biological tissue samples.

An example of the spectral resolution achievable by CP magic-angle spectroscopy is given in Figure 11.2 where the ${}^{13}\text{C}$ natural abundance spectrum is shown for canine Achilles tendon. Tendon was selected as the test material because of its relative molecular homogeneity, i.e., 70-75% collagen, and the high degree of orientational order in the tissue. Under conditions of the experiment (see Figure 11.2 for details), a remarkably well-resolved spectrum is obtained with signal line widths approaching those for biological molecules in solution. Definitive assignments can be made of signals arising from specific amino acid groups, as noted in Figure 11.2. Two particularly distinct, narrow signals centered at 159.0 ppm and 44.0 ppm are unmistakably due to

C_α atoms of arginine/tyrosine and glycine α -carbon atoms, respectively. All of the signals broaden when the spinning axis orientation is set at $\beta = 0^\circ$, with the largest change seen for arginine/tyrosine C_α , consistent with large chemical shift anisotropies for this carbon nucleus.

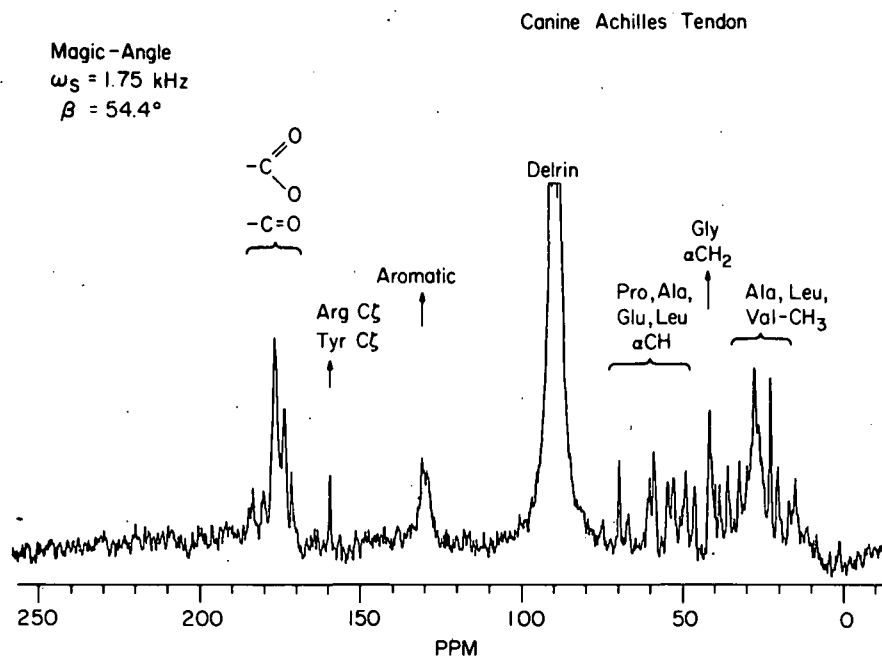


Fig. 11.2. ^{13}C natural abundance spectrum for canine Achilles tendon measured with rapid magic-angle spinning. Tissue samples were tightly packed with the long axis of the tissue vertically oriented in the rotor. The spectrum was recorded at ambient temperature on a Nicolet NT-150 CP spectrometer at a ^{13}C resonance frequency of 37.735 MHz and a constant spinning rate of $\omega_S = 1.75 \text{ kHz}$. A proton dipolar decoupling field $\gamma H_2/2\pi \sim 35 \text{ kHz}$ was used, and the spectrum represents 2,000 accumulated free induction decays. No cross-polarization contact was employed in obtaining the tendon spectrum.

These "range-finding" spectroscopic measurements demonstrate that the means now exist for studying structures of biological molecules directly in tissue, and, by extension, in other biological systems--cells, organs, etc.--with a resolution and precision hitherto possible only for solutions of much smaller organic and biological molecules. Areas where the above approach may be especially useful are in definition of tertiary structures for biological molecules, e.g., proteins, nucleic acids, in whole biological systems, and in comparison of these structures with X-ray crystallographic results. Such studies are currently in progress.

NEW SYNTHETIC USES FOR ADENOSINE N¹-OXIDE DERIVATIVESM. MacCoss, E. K. Ryu, R. S. White,¹ and R. L. Last²

The introduction of novel functionalities into the sugar moiety of nucleoside derivatives is usually accomplished by nucleophilic displacement of suitable leaving groups with an appropriate nucleophile. In the adenosine series, a major problem has been the concomitant formation of N³ → sugar intramolecular cyclized products. The presence of an acyl group at N⁶ decreases the nucleophilicity of N³ and reduces cyclization products but in turn imposes restrictions on the synthetic options available. In the past year, we have shown that N¹-oxides similarly prevent intramolecular cyclization during nucleophilic substitution reactions and that reduction to the parent nucleoside can be readily achieved using Raney nickel or hexachlorodisilane. The synthetic utility

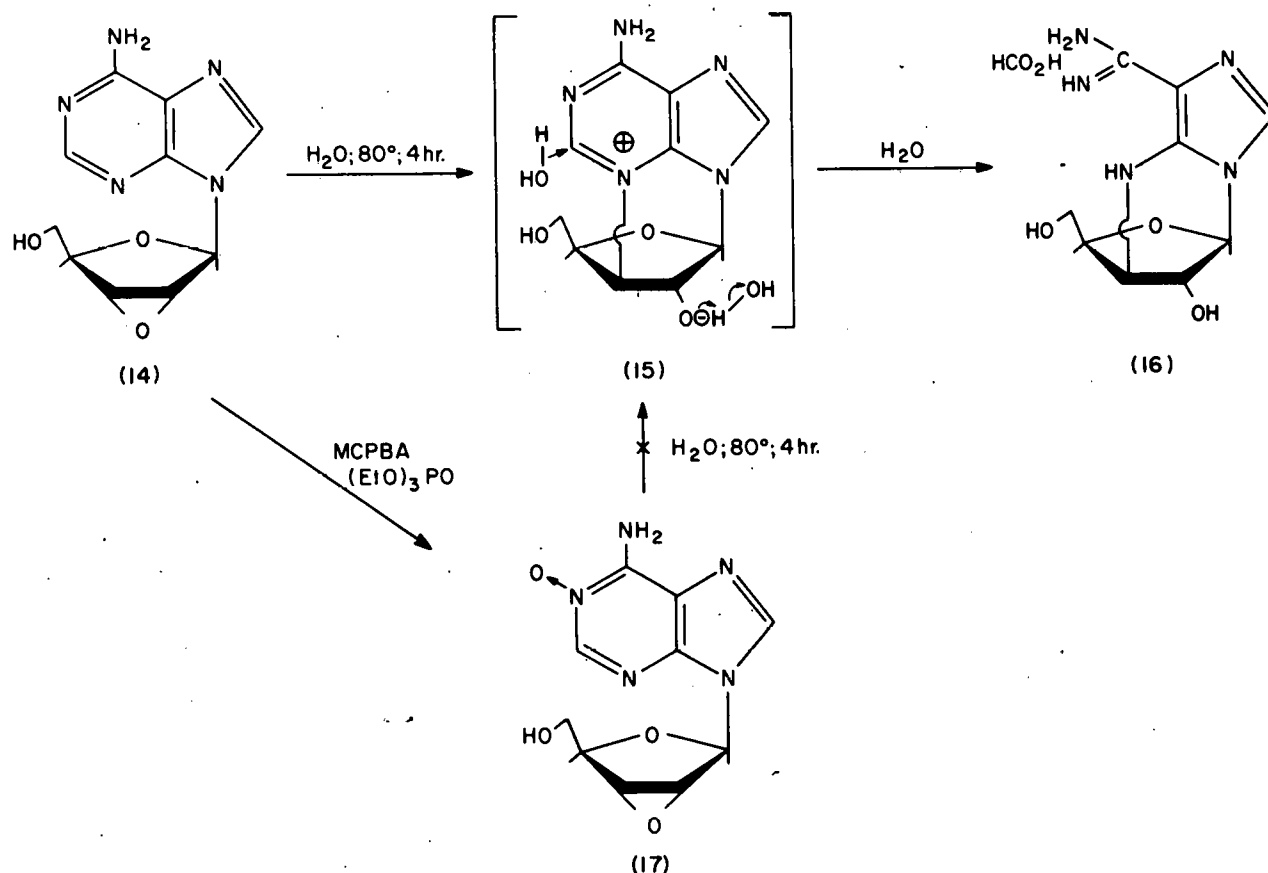


Fig. 11.3.

¹ Spring 1979 participant in the Undergraduate Honors Research Program, Northern Illinois University.

² Fall 1978 participant in the Undergraduate Honors Research Program, Ohio Wesleyan University.

has been demonstrated by the synthesis of 5'-iodo-5'-deoxy-2',3'-O-isopropylideneadenosine-N¹-oxide and 5'-azido-5'-deoxy-2',3'-O-isopropylideneadenosine-N¹-oxide from 5'-O-tosyl-2',3'-O-isopropylideneadenosine-N¹-oxide, and 2',3'-anhydroadenosine-N¹-oxide from 2',3'-anhydroadenosine (see Figures 11.3 and 11.4). In addition, an improved general synthesis of N¹-oxides (using m-chloroperbenzoic acid in triethylphosphate) has been developed.

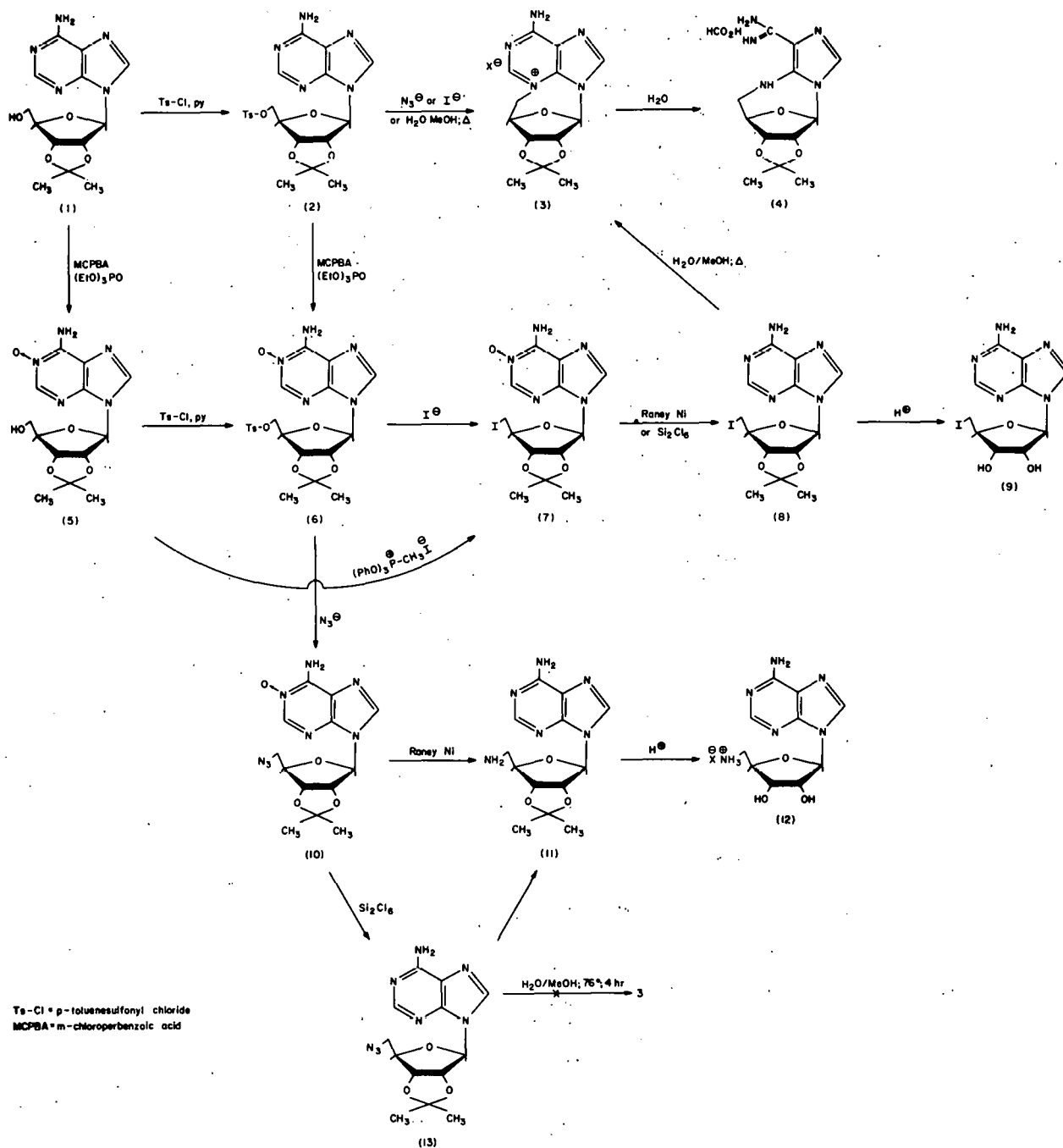


Fig. 11.4

SYNTHESIS OF POTENTIAL ANTIMETABOLITES AND MOLECULAR DEPOT DRUGS

M. MacCoss and E. K. Ryu

In an effort to increase the chemotherapeutic efficacy of the nucleoside analogues arabinocytidine, arabinoadenosine, and tubercidin, these nucleosides have been attached to 1,2-dipalmitin via a pyrophosphate linkage to produce araCDP-dipalmitin (MacCoss, M., et al., Biochem. Biophys. Res. Commun. 85, 714, 1978), araADP-dipalmitin, and TuDP-dipalmitin, respectively. These prodrugs, as sonicated solutions, have been evaluated in the mouse myeloma MCP-11 cell line, in collaboration with T. Matsushita (see Section 14 of this report), and in the L-1210 test system, in collaboration with C. I. Hong, Roswell Park Memorial Institute, Buffalo, NY. Such sonicated solutions of araCDP-dipalmitin and TuDP-dipalmitin showed good activity (Table 11.1) whereas araADP-dipalmitin is insoluble in all solvents so far examined. In addition, careful preincubation studies of the prodrug in the cell culture media and reversal of inhibition using deoxycytidine (in the araCDP-dipalmitin case) have verified that these phospholipid derivatives are acting as prodrugs or "molecular depots" of the parent drug which is then being slowly released by enzymatic hydrolysis. Also, it has been possible to show that release of the drug is occurring intracellularly and not in the culture medium prior to uptake by the cell.

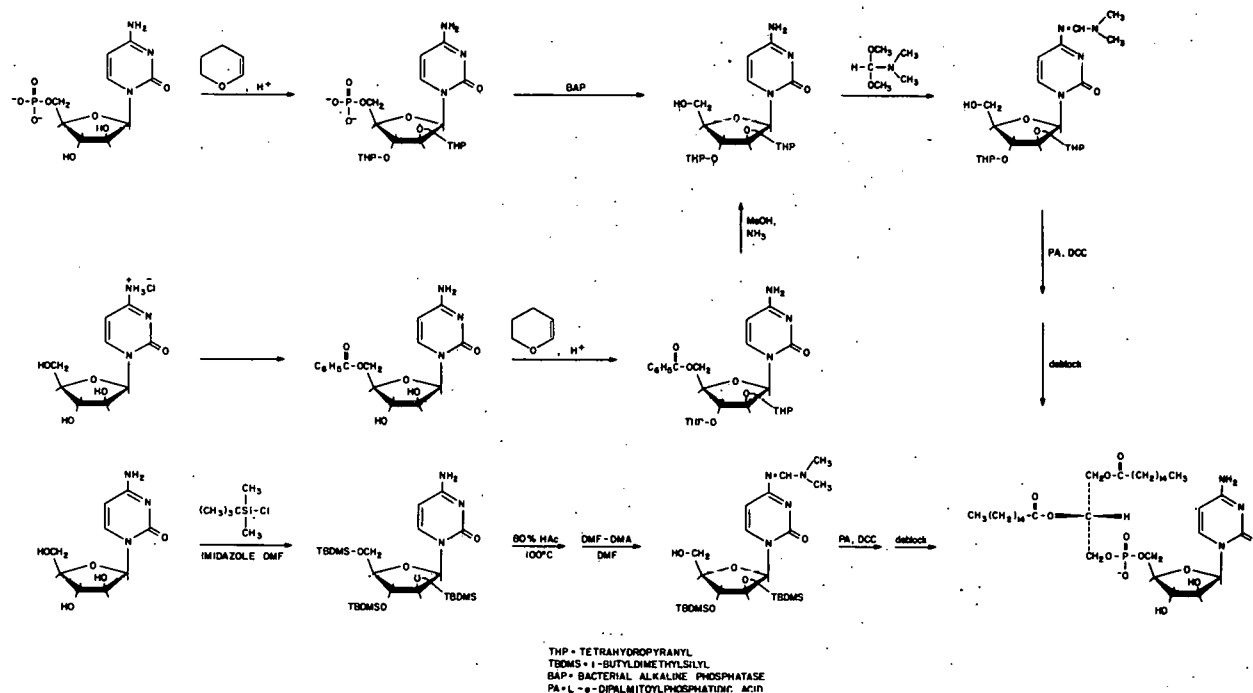


Fig. 11.5.

Table 11.1. Fifty Percent Inhibition of Mouse Myeloma (MPC 11. Subclone 66.2) and L1210 Cell Lines by Selected Nucleoside Analogues

Analyse	ED ₅₀ in Mouse Myeloma Cells ^a	ED ₅₀ in L1210 Cells ^b
araC	1.9 x 10 ⁻⁶ <u>M</u>	1 x 10 ⁻⁷ <u>M</u>
araCMP	3.2 x 10 ⁻⁶ <u>M</u>	5 x 10 ⁻⁸ <u>M</u>
araCDP-L-dipalmitin	5.6 x 10 ⁻⁵ <u>M</u>	4.3 x 10 ⁻⁷ <u>M</u>
Ratio of araC/araCMP values	0.59	2.00
Ratio araC/araCDP-L-dipalmitin	0.03	0.23
Tubercidin	2.6 x 10 ⁻⁷ <u>M</u>	7 x 10 ⁻⁷ <u>M</u>
TubercidinDP-L-dipalmitin	5.0 x 10 ⁻⁶ <u>M</u>	7.7 x 10 ⁻⁶ <u>M</u>
Ratio Tubercidin/TubercidinDP-L-dipalmitin	.05	.09
5'-O-ado-araC	7.8 x 10 ^{-6c} <u>M</u>	-
5'-O-palm-araC	3.5 x 10 ^{-6c} <u>M</u>	-
araC	3.9 x 10 ^{-6c} <u>M</u>	-
araA	9.8 x 10 ^{-5d} <u>M</u>	7 x 10 ⁻⁵ <u>M</u>

^aCarried out by T. Matsushita.

^bCarried out by C. I. Hong, Roswell Park Memorial Institute.

^cCultures grown in 0.6% dimethyl sulfoxide.

^dCultures grown in 0.8% dimethyl sulfoxide.

Much effort has been devoted this year to preparation of monophosphate-linked analogues and several different synthetic approaches have been tried. Two factors must be taken into consideration in the selection of a reaction sequence leading to these derivatives. First, the final condensation must be between phosphatidic acid and a suitably protected nucleoside, since a condensation carried out in the reverse sense (i.e., between dipalmitoylglycerol and a 5'-nucleotide) would lead to migration of the palmitoyl groups and thus lead to a mixture of products. Second, the protecting groups present on the nucleoside portion must be removable under nonbasic conditions, since use of the more commonly used acyl protecting groups would lead to concomitant cleavage of the palmitoyl residues during the deblocking step. With these factors in mind, our initial approaches to the synthesis of monophosphate-linked derivatives are shown in Figure 11.5. Similar schemes have been attempted for

arabinoadenosine. The approach currently being followed utilizes the tetrahydropyranyl (THP) group to protect the 2'- and 3'-OH groups (see Figure 11.5) since removal of the t-butyldimethylsilyl (TBDMS) group from the final product (using $\text{Bu}_4\text{N}^+\text{F}^-$) gave unexpected degradation products.

The predicted advantages of phospholipid derivatives as prodrugs include (1) potential for targeting to specific tissue, (2) potential for activity against resistant cell lines, and (3) resistance to catabolizing enzymes.

CRYSTALLOGRAPHIC REFINEMENT OF THE Mcg λ -TYPE BENCE-JONES DIMER

M. Schiffer

The principal objective of the refinement is to obtain the most accurate coordinates for the more than 3,000 nonhydrogen atoms derivable from the diffraction data of the Bence-Jones dimer. Accurate 3-D structures are essential for comparison of structural features such as the immunoglobulin fold with those of other immunoglobulin fragments. The refinement will aid in the correlation of the amino acid sequence with the binding site conformation. It will also facilitate the use of the Mcg protein as a search structure to determine structures of other immunoglobulin fragments by molecular replacement methods.

The crystallographic refinement is the last phase of the structural study. Up to now we have used the method of "constrained crystallographic refinement." The resulting molecular model was then compared with the calculated electron density map (see Figure 11.6). Manual changes in the model were made with the aid of an interactive computer graphics system, GRIP-75,¹ at the University of North Carolina. To get a better fit to the last map examined, the positions of 31 residues were adjusted; this required altering the main chain orientation in those positions. Electron density was visible for 19 more atoms of the structure. The conformations of C-terminal residues 215 and 216, including the disulfide bond connecting the two monomers, were questionable and therefore left out of subsequent calculations. Their position will be determined from difference Fourier maps after further refinement. Although the refinement improved the quality of the electron density map, no density is yet seen for 205 out of the 3222 nonhydrogen atoms of the molecule. These atoms are part of side chains such as lysine on the surface. Also, there is very little electron density for one of the hypervariable loops, which might be a characteristic of the structure.

Following the incorporation of the above changes, four cycles of refinement brought the R-factor to 29.5%.

¹GRIP-75 developers included E. G. Britton, F. P. Brooks, Jr., J. Hermans, J. S. Lipscomb, J. E. McQueen, M. E. Pique, and W. V. Wright.

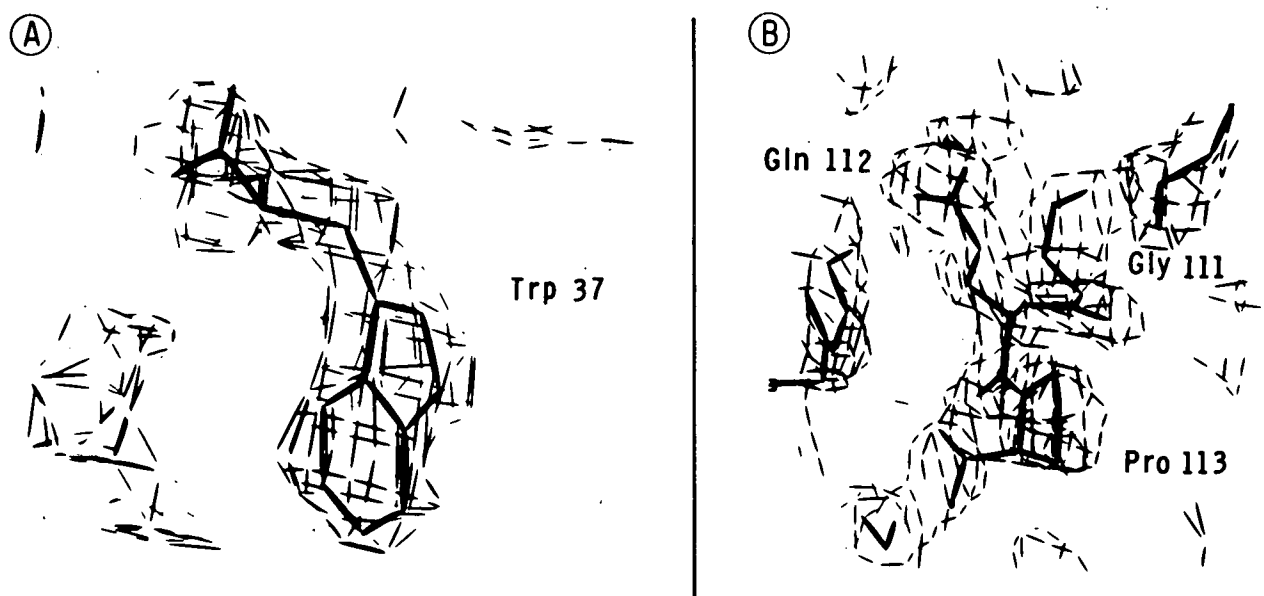


Fig. 11.6. A and B, examples of fit of sections the molecular model (dark lines) to the calculated electron density envelope. Photographs taken at the University of North Carolina.

STRUCTURE DETERMINATION OF THE κ_{II} -TYPE BENCE-JONES PROTEIN FIN

M. Schiffer, N. Panagiotopoulos,¹ F. A. Westholm, F. J. Stevens, and A. Solomon²

Structure determination has started on the protein Fin, the first complete κ -type Bence-Jones protein ever crystallized. Solving the structure of this protein is of special interest because Fin is a complete κ -chain consisting of two noncovalently bound monomers and because it is a light chain of a κ -chain subgroup, κ_{II} . This subgroup is characterized by proteins possessing an insertion of up to six amino acid residues between positions 29 and 30 in the first hypervariable region. Crystals of the protein Fin obtained from ammonium sulfate solutions have the orthorhombic space group $P2_12_12_1$ with cell dimensions $a = 132.0$ Å, $b = 93.3$ Å, and $c = 42.3$ Å. The asymmetric unit consists of a dimer of molecular weight $\sim 46,000$ daltons and the diffraction pattern extends to 2.7 Å spacing (Schiffer, M., et al., ANL-78-90, 1978, p. 180).

¹Nuclear Research Center "Demokritos," Athens, Greece.

²Memorial Research Center, University of Tennessee, Knoxville.

Molecular replacement methods were applied in an attempt to determine the structure of the Fin protein. Crowter's rotation function with 6 Å data was used to locate the orientation of the variable and constant regions of the Fin molecule. Rei V fragment dimer (κ_I) and C dimer of Mcg λ chain were used as search structures after the residues, which are expected to be different in the Fin molecule, were removed. Possible orientations for both variable (V) and constant (C) regions were found. Both rotation peaks required by the twofold axis of the V and C regions were located. The angle formed by the twofold axis of the V region with the twofold axis of the C region is either 106° or 140°, depending on which symmetry equivalent rotation peak is chosen. These angles compare well with the equivalent angle of the Mcg dimer (113°) and the McPC 603 Fab fragment (135°).

SELF-ASSOCIATION OF κ_I -IMMUNOGLOBULIN LIGHT CHAINS

F. J. Stevens, F. A. Westholm, A. Solomon,¹ and M. Schiffer

Bence-Jones proteins are immunoglobulin light chains found in the urine of patients with multiple myeloma. The κ_I proteins, a subgroup of the Bence-Jones proteins, constitute the largest single group of light chains, representing about 35-40% of the light chains in human sera. Because there is better than 70% homology among the amino acid sequences of the different κ_I chains, a comparison of the association properties of light chains from the κ_I subgroup is of particular interest, as differences must result from relatively few changes in primary structure.

Gel electrophoresis and molecular sieve chromatography were used to compare 14 different κ_I -type Bence-Jones proteins including two for which the amino acid sequence is known. Although electrophoresis in the presence of sodium dodecyl sulfate found uniformity of covalent dimer and monomer molecular weights, Sephadex chromatography under nondissociating conditions showed monomers to elute with different apparent molecular weights. The apparent molecular weight was dependent on protein concentrations. Computer simulation of associating systems during molecular sieve chromatography was used to understand the behavior of these proteins. Based on the simulation, it was found that the dimerization constants of the light chains studied varied from 10^6 M^{-1} to less than 10^3 M^{-1} and therefore that this variability was the cause of the observed differences in apparent molecular weights.

The variable region and more specifically the third hypervariable region appear responsible for this variation in dimerization constant. Association properties of light chains of known sequence suggest that the presence of an aromatic or hydrophobic residue at position 96 enhances dimer formation, while a charged residue at that position results in inhibition of dimer formation in light chains. Hypervariable residue 96 may be at the beginning of the J segment. If so, this segment may be one of the sources of the variability of self association of light chains.

¹Memorial Research Center, University of Tennessee, Knoxville.

DESIGN AND DEVELOPMENT OF A SMALL-ANGLE DIFFRACTOMETER FOR A PULSED NEUTRON SOURCE

C. Borso

A large-scale multidivisional program is under way at Argonne National Laboratory to develop an intense pulsed spallation neutron source (IPNS-1) suitable for studies in condensed states including biological systems. This facility is scheduled to become operational in 1981 and will provide thermal-neutron fluxes of 10^{15} neutrons per square centimeter per second. A prototype of this source, i.e., ZING-P', which became operational in FY 1979, has permitted beam measurements and design studies for a variety of instruments to be incorporated at IPNS-1.

Initial design studies for a small angle neutron scattering (SANS) diffractometer indicated that an effective high resolution time of flight SANS diffractometer could be constructed at the proposed IPNS facilities. The instrument will feature a converging slit collimator and 20 x 20 cm two-dimensional position sensitive detector and has a wide range of accessible Q values with approximately constant resolution over most of the range.

In order to develop final design concepts and project performance characteristics for the IPNS-1 instrument, design and construction was undertaken of a prototype SANS diffractometer for ZING-P', incorporating time of flight principles, and optimized for scattering studies on biological systems. Factors taken into account included physical limitations imposed by the target area, moderator, shielding, and experimental facilities, as well as limitations imposed by the source spectrum.

Using seven tapered Söller collimators, as illustrated in Figure 11.7, a SANS instrument coupled to ZING-P' is projected to have the following characteristics:

- ϕ = total flux through sample = 1.4×10^5 neutrons/second
- Q_{\min} = minimum wavevector = $3.8 \times 10^{-3} \text{ \AA}^{-1}$
- Q_{\max} = maximum wavevector = $2.6 \times 10^{-1} \text{ \AA}^{-1}$
- $\Delta\theta$ = angular resolution = 2.0 milliradians

These characteristics are based upon utilizing a two-dimensional position sensitive detector (17 x 17 cm in size, with a spatial resolution of 5 mm) which is patterned after the Oak Ridge detector and which is currently undergoing testing by the ANL Chemistry Division. The characteristics enable investigation of biological macromolecules in the range of 10,000 to 250,000 daltons. If this prototype meets the above design specifications, the expected increase in flux for the IPNS-1 instrument opens the way for a higher resolution SANS diffractometer extending the range of investigation to macromolecules from 10,000 to 2,500,000 daltons.

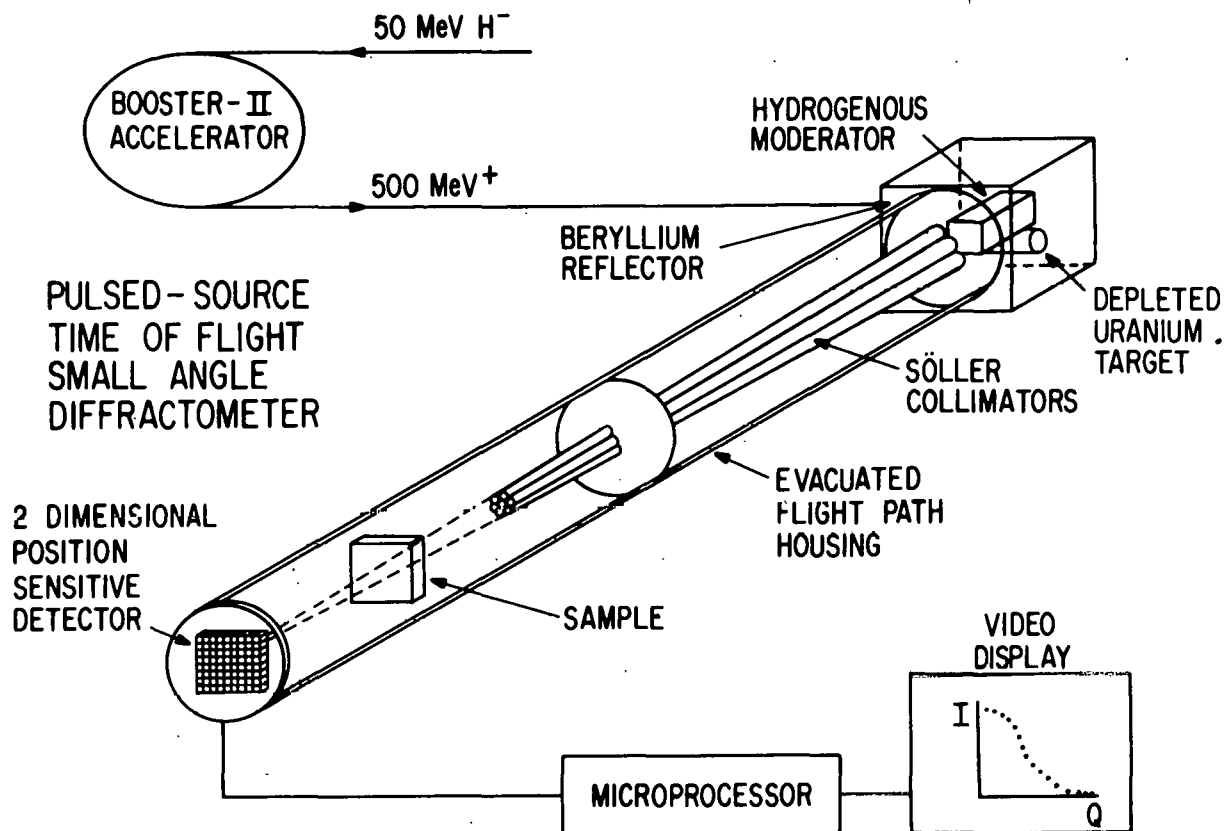


Fig. 11.7. Söller collimator design for the ZING-P¹ neutron small angle diffractometer.

CIRCADIAN PHYSIOLOGICAL MANIFESTATIONS OF BARBITURATE ADDICTION AND WITHDRAWAL IN THE RAT

C. F. Ehret, C. Peraino,¹ J. C. Meinert, and K. R. Groh

When administered in a punctate fashion, pentobarbital is a chronobiotic, i.e., it phase-shifts chronotypically the circadian rhythm (Ehret, C. F., et al., *Science* 188, 1212, 1975). Chronic administration of dietary phenobarbital (0.3%) in the absence of other temporal cues results in dyschronism, i.e., the loss of circadian synchrony, within the first 5 or 6 days of the start of the habit (Ehret, C. F., and K. W. Dobra, in *Proceedings of the Third International Symposium on the Detection and Prevention of Cancer*, H. E. Nieburgs, ed., Marcel Dekker, New York, 1977, pp. 1101-1114). The

¹Carcinogenesis Group.

present study extends these observations to include diverse habituation and drug ingestion circumstances, including a comparison of the effect of pre-entrainment cycles (FS 7:17, DL 17:7 vs FS 12:12, DL 12:12)¹ and subsequent conditions of exposure (FF, DL 12:12 vs FF, DD and FF, DL 17:7) of duration sufficient to permit a "steady-state" in the appearance of circadian patterns derived from core temperature telemetry and from automated food consumption measurements. Finally, measurements were permitted to continue long enough after the drug was withdrawn from the diet to allow characterization of an abstinence syndrome, indicative of addiction.

In nearly every case following the ingestion of dietary phenobarbital (0.25% in this series), marginal or total dyschronism was observed for 2 to 5 days; this was much more acute in rats in continuous dim light than in DL rats. The latter, which had been entrained by 12:12 dark light cycles, readily achieved a "steady state" circadian oscillation of body temperature. The wave form, however, was dramatically distorted by an increase in amplitude (from 0.7°C to 1.2°C) and by a phase angle difference (Ψ advance) of about 32 degrees, equivalent to an advance of 2.1 hours in the thermal acrophase. This wave pattern then continued even in the absence of a DL zeitgeber (i.e., during DDFF) for 8 days. Similar behavior was seen in rats entrained by DL 17:7 illumination cycles. With this reduced phase ratio for the zeitgeber, few Ψ shifts occurred, and these were smaller than those induced in the DL 12:12 group. In each group, withdrawal of the drug from the diet resulted in an abstinence syndrome of severe dyschronism for 3-6 days even during DL entrainment, before normal circadian patterns were restored. For example, Figure 11.8 shows the dyschronogenic effect of phenobarbital withdrawal on seven rats that had been on dietary phenobarbital for 30 days. Thus, a complete barbiturate effect, with habituation and a withdrawal sequence, is observable from circadian criteria: (1) the chronobiotic or punctate zeitgeber effect upon phase and phase response; (2) the early chronic effect, dyschronogenesis, recognized as a transient of long duration; (3) the steady state effect, with amplitude enhancements and Ψ shifts dependent in magnitude upon phase ratio of the zeitgeber; and (4) an abstinence syndrome characterized by severe dyschronism when the drug is withdrawn. A more detailed view of one portion of this work from the perspective of the cocarcinogenic action of dietary phenobarbital is presented in the first report by Peraino et al. in Section 14.

¹Abbreviations: FS, feed-starve cycle; DL, dim light-bright light cycle; FF, continuous feeding (*ad libitum*); DD, continuous dim light. Ratios indicate the hours per day on the regimens noted.

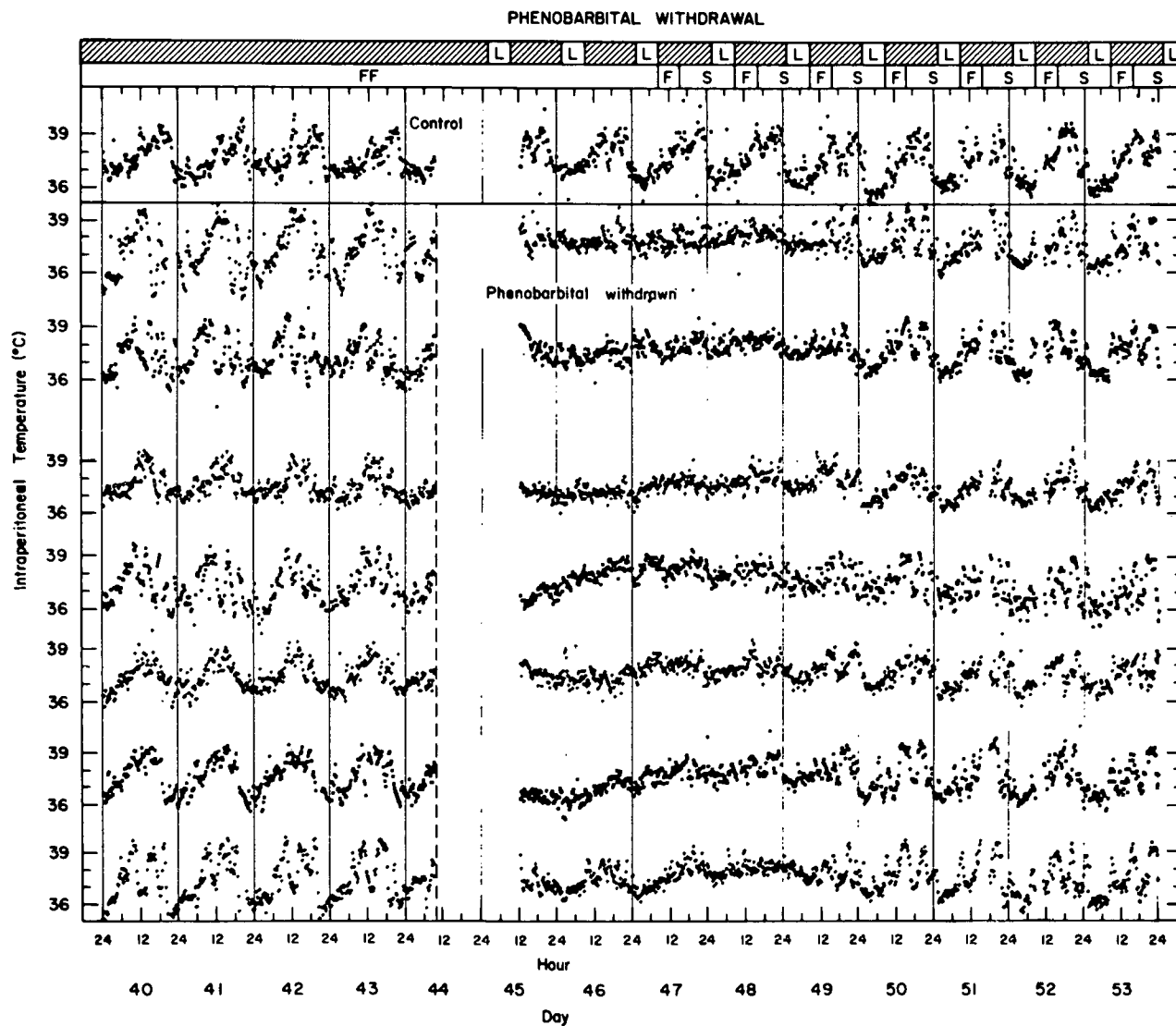


Fig. 11.8. Intraperitoneal temperature measured every 15 minutes for eight animals over 14 days. The regimens for lighting and feeding are given at the top. L = light phase from 0200-0900 hours, F = food available, S = food withdrawn. The top animal is a control and the remaining seven animals were exposed to phenobarbital in the diet at a concentration of 0.25%. The phenobarbital diet was removed at the dashed line when feeding on the control diet began.

CIRCADIAN REGULATION ALONG THE NEUROENDOCRINE AXIS

C. F. Ehret, A. L. Cahill,¹ N. D. Horseman,¹ K. R. Groh, and
J. C. Meinert

A new approach to our continuing study of circadian regulation was begun in June 1978. Of particular interest are the roles of catecholamines in circadian and infradian decision making and their interplay with hormones of the adrenal cortex and the anterior pituitary. Although coordination of infradian organismic functions by hormonal action in synchrony with a circadian oscillator has been assumed throughout the literature, experimental demonstration of such control is lacking. In our present studies, preliminary results clearly show that the glucocorticoid dexamethasone is a circadian zeitgeber in the rat. When the hormone is administered intraperitoneally (10 mg/kg) at various phases of the circadian cycle to rats fed ad libitum, and maintained in continuous dim light, a typical phase response curve can be drawn from the resultant phase advances and phase delays; an insensitive phase corresponds to the normal peak of the corticoid circadian rhythm, and phase delays precede phase advances. Similar studies with the pituitary hormone prolactin have also begun.

In the last quarter of the year, high performance liquid chromatography techniques for the quantitation of catecholamines, indoleamines, and their metabolites in biological samples were implemented in the laboratory. Using an electrochemical detector it was possible to quantitate as little as 0.5 ng of each of the separated amines: norepinephrine, epinephrine, dopamine, and 5-hydroxytryptamine. In terms of brain levels, this corresponds to the ability to detect amine concentrations of about 0.1 µg/g in as little as 80 mg of wet brain tissue. The ability to quantitate low levels of these amines and their metabolites is essential to the planned studies on the regulation of the observed circadian fluctuations in catecholamine and indoleamine content of the mammalian central nervous system.

Studies complementary to those described above at the organismic level have been resumed at the cellular level. These studies, which measure direct cellular responses, are aimed at distinguishing interactions between hormones and "master oscillators" in the intact organism. We have perfected the techniques for isolating rat hepatocyte cell lines, and one entire unit of an automated circadian respirometer became operational. Pilot studies with hepatocytes in vitro in the circadian respirometer have shown that, as in Tetrahymena, infradian respiration is photosensitive.

¹Biomedical Effects of Energy Transmission Group.

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12. MUTAGENESIS: MOLECULAR AND GENETIC MECHANISMS OF ENVIRONMENTAL MUTAGENS

INTRODUCTION

Herbert E. Kubitschek

The production of genetic (DNA) lesions is a major deleterious effect of chronic exposure of living cells to pollutants. Genetic lesions are responsible for serious heritable disorders in man and they are generally believed to be responsible for cancer. It has been estimated by the International Agency for Research on Cancer that environmental mutagens cause as many as 80% of cancers.

Because environmental mutagens act by producing DNA lesions, this genetics program is concerned primarily with the kinds of DNA lesions produced by environmental and energy-related mutagens, the mechanisms of action of these lesions, and their repair. This information is vital to the rational development of improved mutagen testing systems for assessment of human risk and to the development of procedures of reducing mutational damage.

Bacteria are used in our studies of DNA lesions and mechanisms of mutation at the molecular level. These organisms provide by far the best mutational system because of the great amount of information gathered on them in previous mutation studies, their exceptional convenience for laboratory studies, and the fact that their genetic maps are much more complete than those for any other organism. Mouse myeloma cells also are used because these mammalian cells are particularly convenient for short-term cytotoxic and cytogenetic studies and because these cells may have mutagen sensitivities that are likely to be more similar to those in man.

A second major endeavor concerns the improvement of bacterial and mouse myeloma cell systems to assays of biological risk from exposure to effluents of coal technologies. This work includes examination of ongoing testing systems to determine limits of their applicability, and the ongoing development of new and/or improved rapid screening systems.

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MUTATIONAL SYNERGISM

H. E. Kubitschek and D. Venters

Many environmental and energy-related pollutants are currently being examined in rapid screening tests with bacteria in many laboratories. The standard procedure is to test pollutants individually. However, such tests may greatly overestimate or underestimate the effects of mutagens when they are applied simultaneously, either because of chemical interactions between the mutagens, destruction of DNA repair enzymes, or because of interactions at the genetic level. We are concerned here with the latter possibility, synergism in the induction of mutation, which can arise whenever mutagenesis occurs as a dose-squared function. We have demonstrated that synergism does occur between two particular mutagens that provide a dose-squared response in Escherichia coli WP2s, namely, ultraviolet light and nitrous acid. Mutant frequencies were increased by 70% after exposure to both of these mutagens, as compared to the sum of the individual exposures.

If synergism occurs at the genetic level rather than through destruction of repair enzymes, then the degree of synergism should be independent of the order in which the two mutagens are applied. We have determined that this is indeed the case for the synergistic interaction between ultraviolet light and nitrous acid.

This support for induction synergism has led to the development of a mathematical model for mutagens that individually follow dose-squared responses. Theoretically, strong mutagens can produce up to 100% more mutations than expected from their individual actions. However, weak mutagens have no such limitation. Induction synergism between two or more weak mutagens may produce many more mutations than could be accounted for by their individual actions.

REPRODUCIBILITY OF THE AMES SALMONELLA MICROSOMAL ASSAY

H. E. Kubitschek and D. M. Williams

The Ames Salmonella microsomal assay system is a standard screening system used in laboratories throughout the nation as a prescreen for potential carcinogens and for information on the relative mutagenicity of environmental pollutants. The assay system is rapid, inexpensive, and gives values for mutagenic potency that are in good correlation with carcinogenic potency. The variability that we observed between daily tests with this system, however, led us to examine its reproducibility.

In order to avoid possible variability introduced by use of the liver microsomal (S-9) enzyme activation systems, we examined test reproducibility for mutagens that did not require activation. The tests were carried out precisely as prescribed by Ames and co-workers (Ames, B. N., et al., Mutat. Res. 31, 347, 1975) with the exception that the same bacterial culture of Salmonella

typhimurium TA98 was employed in each test. Aliquots of this culture were withdrawn from the liquid air chest (-190°C) just prior to testing.

In an initial series of tests, a standard sample of 2-nitrofluorene in dimethyl sulfoxide was assayed in triplicate daily at 0.1, 0.2, and/or 0.4 $\mu\text{g}/\text{plate}$. The average number of revertants per plate was essentially proportional to the amount of nitrofluorene per plate. However, the variability was quite large; the coefficient of variation was about 33%, which was much greater than the expected (Poisson) variability of about 4%. In a second series of tests, a standard dimethyl sulfoxide extract of particulate effluent from a pressurized fluidized bed combustor (Sample A, Table 12.1) was plated independently by three persons and linear responses were obtained. The coefficient of variation of these responses, fitted by least squares, also was large, 32%. Sample B, an acetone/methanol condensate of the same material, also had about the same coefficient of variation, 35%. Thus, for each of the three mutagens tested, the variability was observed to be between 32% and 35%.

Although the source of this intrinsic variability is as yet undetermined, the results indicate that accuracy requires repeated application of the Ames test. To reduce errors to 10%, for example, the test would have to be performed approximately 10 times. Thus estimates of the expense of the test for quantitative applications must be greatly increased.

Table 12.1. Ames Assay Variability Determined from Linear Regressions of Responses of *Salmonella* Strain TA98 without Metabolic Activation to Two Mutagenic Extracts

No. ^c	Sample A ^a		Sample B ^b		Mutagenicity Ratio ^e	CV
	Mutants/mg	CV ^d	Mutants/mg	CV		
3	288	18%	7,500	30%	26	27%
2	278	18%	5,100	42%	18	26%
3	420	30%	10,000	31%	24	10%
Averages	335	32%	8,300	35%		23%

^a Dimethyl sulfoxide extract of particulate effluent from an experimental fluidized bed coal combustor.

^b Acetone/methanol extract of the same material used in Sample A.

^c Number of trials by each different investigator.

^d Coefficient of variation.

^e Ratio of values of Sample B to Sample A.

DELAYED REPLICATION OF DIFFERENTIALLY STAINED CHROMOSOMES IN MOUSE MYELOMA CELLS

T. Matsushita, A. Simms,¹ and G. Matsushita²

The bromodeoxyuridine (BUdR) differential staining technique has mainly been used to measure sister chromatid exchanges, such as those induced by carcinogens and mutagens. However, this technique can also be used to measure DNA replication kinetics. We have adapted the BUdR technique to mouse myeloma cells to study the delay in DNA replication induced by far ultraviolet light (95% 254 nm). A delay of 5 hours was observed with a dose of 7.5 J m^{-2} . Parallel growth analysis with a Coulter counter showed an identical delay of 5 hours in growth. Both replication and delay values were independent of parent culture doubling times. After the delay period, irradiated cultures resumed replication at a rate similar to that of the control. These kinetic data suggest that UV-induced damage causes an initial delay in DNA replication but that the persistent DNA damage that remains (pyrimidine dimers) does not affect either the DNA replication or cell division processes.

CELL GROWTH AND PYRIMIDINE DIMER REMOVAL IN MOUSE MYELOMA CELLS

T. Matsushita and A. M. Shotola

Past studies of pyrimidine dimer removal have shown that mouse cells remove only 10% of the dimers in their DNA within 24 hours compared to 70% in human cells; yet the human cells are more sensitive to far ultraviolet light (UV) than mouse cells. In an effort to determine the significance of this limited removal in mouse cells, we have measured the removal of dimers relative to growth curve position rather than time. These studies reveal two new facts, not obtainable from time course kinetics: (1) There are two classes of dimers; one class (35%) was removed during the first generation of growth after irradiation while the other (65%) was not excised for at least three generations of growth. (2) The partial excision of dimers (35% loss) begins only after cell numbers start increasing. That is, dimers are not removed until the cells are competent to undergo cell division, presumably when the cell has completed repair of lethal damage. These data indicate either that dimers are not lethal lesions in mouse myeloma cells, or that cells can cope with dimers by other DNA repair processes without removing the dimer itself.

¹Sponsored by the Argonne Affirmative Action Program.

²Cytogenetic consultant.

ELECTRICAL DETERMINATION OF VIABILITY IN MOUSE MYELOMA CELLS

T. Matsushita, A. M. Brendzel,¹ and A. M. Shotola

Suspension of mouse myeloma cells in phosphate-buffered saline (PBS) was found to induce a significant amount of cell death. Cell death was initially detected with a Coulter counter as the increase in the proportion of cells of smaller size, as determined electrically. The accuracy of this method was confirmed by trypan blue exclusion and soft agar cloning. Comparison of a mixture of live and dead cells by electrical sizing and by trypan blue exclusion showed a high correlation between the results of the two techniques (correlation coefficient: 0.98). Sizing of PBS-treated cells by light microscopy suggests that the altered electrical size distribution (ESD) of the PBS-treated cells was due to decreased cell volume as well as increased permeability of cell membranes. To examine the generality of ESD changes as an indicator of lethality, heat-killed cells were also measured on the Coulter counter. In contrast to the earlier results, the ESD of heat-killed cells did not differ significantly from that of control cells. Light microscopy studies confirmed the same size distribution for heat-killed cells and unheated control cells. We conclude that electrical sizing is an effective method for determining viability of mouse myeloma cells when cell death is accompanied by a change in cell size, as in PBS-killed cells, or when cell membrane permeability changes significantly.

MOUSE MYELOMA SUSPENSION CULTURES AS A SYSTEM FOR CYTOTOXIC AND CYTOGENETIC TESTS FOR ENVIRONMENTAL POLLUTANTS

T. Matsushita and G. Matsushita²

Mouse myeloma cells are conveniently grown in suspension cultures and have a moderately short generation time of 16 hours. Two rapid methods, requiring only 2 to 3 days, have been developed using these mammalian cells to screen for potentially hazardous pollutants: (1) the suspension culture cytotoxicity test, and (2) the sister chromatid exchange test. The application of the two tests provides a rapid system for combined cytogenetic and cytotoxic effects.

Suspension Culture Cytotoxicity

Using the electronic cell counter method described in the preceding report for distinguishing between live and dead cells, accurate growth curve and lethality measurements can be performed electronically on the same culture, obviating the need for laborious and less accurate microscopic and plating techniques. Suspension cultures also enable accurate, multiple sampling

¹Laboratory Graduate Program Participant, University of Illinois at Chicago Circle.

²Cytogenetic consultant.

from a single parent culture, in contrast to the less accurate parallel platings required in monolayer attached cell culture systems to develop kinetic growth curves. Cytotoxic effects on cell growth also can be conveniently determined with a considerable saving of time over that required by attached cell systems. Representative results are shown in Table 12.2, in which four different condensates from the effluent stream of fluidized bed coal combustion are compared for two cytotoxic end points, growth inhibition (ED_{50}) and lethality (LD_{50}). These results were obtained with one parent culture, and compare favorably with test results obtained with other eukaryotic systems.

Table 12.2. Cytotoxicity of Coal Combustion Condensates for Mouse Myeloma Cells^a

Condensate	ED_{50}	LD_{50}	LD_{50}/ED_{50}
FBC-60	8.3	14.7	1.77
Fraction 1	7.7	14.5	1.88
Fraction 2	5.4	12.8	2.37
Fraction 3	13.0	27.5	2.12
Average 2.04 ± 0.27 SD			

^aSamples were from the effluent stream of the experimental bench scale pressurized fluidized bed combustor in the Chemical Engineering Division. All values are in $\mu\text{g/ml}$. ED_{50} is the condensate concentration causing 50% inhibition of cell growth relative to control cultures with no condensate and LD_{50} is the concentration causing 50% lethality relative to control cultures.

Sister Chromatid Exchange Test

This test, developed with other cell lines, has been adapted to suspension cultures of mouse myeloma cells. In contrast to chromosome aberrations, which can be induced by nonmutagens, sister chromatid exchanges appear to be induced specifically by mutagens, and are extremely sensitive. Preliminary results indicate that both cytotoxic and cytogenetic effects of chemical mutagens (e.g., methyl methanesulfonate), antitumor drugs (e.g., arabinofuranosylcytosine), coal combustion effluents, coal conversion process streams, and far ultraviolet irradiation can be determined easily and reproducibly in mouse myeloma cells.

NEAR-ULTRAVIOLET MUTAGENESIS: THE ROLE OF recA⁺ lexA⁺ REPAIR IN MUTAGENESIS BY MONOCHROMATIC 365-nm AND BROAD SPECTRUM RADIATION

R. B. Webb

Radiation at 365 nm is mutagenic for reversion to tryptophan independence in stationary-phase cells of *Escherichia coli* strains WP2s (uvrA) (Webb, R. B., J. Bacteriol. 133, 860, 1978), WP2 (wild type), WP6 (polA uvrA⁺), and WP6s (polA uvrA) when the mutant frequency is assayed on minimal plates supplemented with 2% (v/v) nutrient broth (SEM plates) (Figure 12.1, Part A). High fluence rates (500-1500 W m⁻²) at 0°C effectively prevent concomitant photoreactivation (Brown, M. S., and R. B. Webb, Mutat. Res. 15, 348, 1972). Tryptophan revertants were below detection in strains WP5 (lexA), WP10 (recA), and WP100 (uvrA recA), implicating recA⁺ lexA⁺-dependent error-prone ("SOS") repair in near-UV mutagenesis. Both oxygen-independent (pyrimidine dimers) and oxygen-dependent DNA lesions are implicated in near-UV mutagenesis by the photoreactivation of premutational lesions and the complex oxygen dependence of mutagenesis shown by the strains that were mutated. In contrast to the results with monochromatic 365-nm radiation, only the uvrA strains WP2s and WP6s were mutated significantly by high fluence rate (50-100 W m⁻²) fluorescent BLB (310-405 nm) radiation (Figure 12.1, Part B).

Mutant frequency-fluence relationships with 254-nm radiation in the recA⁺ lexA⁺ strains followed slopes of approximately 2 on a log-log plot. However, the same strains and experimental conditions revealed complex mutant-frequency responses at 365 nm: at relatively low fluences, the mutant-frequency response was linear; at higher fluences, the response followed a slope of 2.5 with WP2s and WP6s, 4.5 with WP6, and 6.5 with WP2.

The absence of broad spectrum near-UV (BLB) mutagenesis in recA⁺ lexA⁺ uvrA⁺ strains (WP2 and WP6) that are strongly mutated at 254 and 365 nm cannot be accounted for either by the absence of pyrimidine dimer formation or concomitant photoreactivation of dimers (data not shown). The low level of BLB mutagenesis observed in the excision-proficient strains WP2 (polA⁺ uvrA⁺) and WP6 (polA uvrA⁺), in contrast to the high level of mutagenesis observed in the excision-deficient strains WP2s (polA⁺ uvrA) and WP6s (polA uvrA), indicates that the uvrA endonuclease is associated with the low levels of BLB mutagenesis obtained in strains that are mutated by 254-nm radiation. The presence or absence of polymerase I did not substantially alter BLB mutagenesis. The near-UV mutational response obtained is consistent with selective damage to components of DNA repair. It is proposed that BLB radiation differentially inhibits the error-prone pathways of DNA in wild-type strains, thereby strongly reducing mutant frequencies in strains capable of error-free repair.

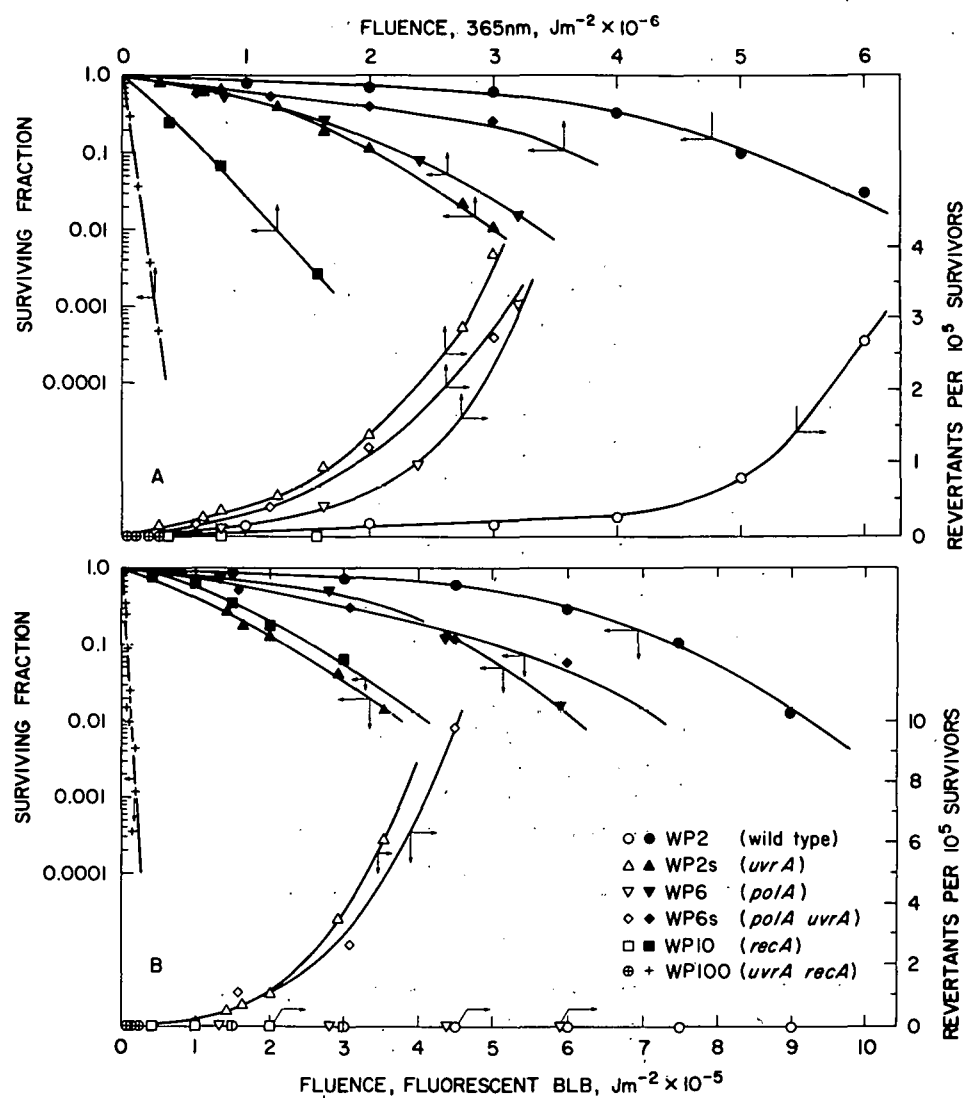


Fig. 12.1. Mutagenesis (*trp* *trp*⁺) and lethal response of seven genetically related strains derived from *E. coli* B/r that differ in repair capability with monochromatic 365-nm radiation (Part A) or fluorescent BLB (310-405 nm) radiation (Part B). All mutant and lethal assays were done on minimal glucose agar plates supplemented with 2% nutrient broth. Fluence rates were 800-1200 W m^{-2} with 365-nm radiation and 60-85 W m^{-2} with BLB radiation. Short wavelength stray light was reduced with a Corning 0-52 filter (1/2 thickness) (1% transmittance 336 nm). BLB radiation was filtered only with 3 mm of Pyrex.

SYNERGISTIC AND ANTAGONISTIC RELATIONSHIPS BETWEEN BROAD SPECTRUM NEAR-UV AND FAR-UV RADIATION IN STRAINS OF ESCHERICHIA COLI WITH DIFFERING REPAIR CAPABILITIES

M. A. Turner¹ and R. B. Webb

Interaction of broad spectrum near-UV (fluorescent BLB) and far-UV (254 nm) radiation was studied in Escherichia coli WP2 (wild type), WP2s (uvrA), WP10 (recA), WP6 (polA), WP6s (polA uvrA), and WP100 (uvrA recA). BLB irradiations were carried out at 0°C at fluence rates of approximately 70 W m⁻² to reduce concomitant photoreactivation to a minimum. Mutation induction (reversion to tryptophan independence) was large with 254 and 365 nm radiations for strains WP2, WP2s, WP6, and WP6s, whereas with broad spectrum BLB radiation, only WP2s and WP6s demonstrated significant mutagenesis (R. B. Webb; see preceding report). The hypothesis that BLB radiation selectively inhibits error-prone recA lexA repair was tested by sequential fluorescent BLB-254 nm irradiation of stationary-phase populations. With strain WP2, a fluence of 30 J m⁻² at 254 nm induced 15×10^{-8} trp⁺ mutants. However, when 1×10^5 J m⁻² or more of BLB radiation preceded the 254-nm exposure, no trp⁺ revertants could be detected (Figure 12.2, Part A). In contrast, when 254-nm irradiation (1 J m^{-2}) was preceded by an exposure to BLB radiation ($5\text{--}50 \times 10^4 \text{ J m}^{-2}$) with strains WP2s (uvrA) and WP6s (polA uvrA), the revertant yield increased sharply (Figure 12.2, Part B). These results are consistent with our previous studies which demonstrated the inhibition of excision, postreplication, and photoreactivation repair by monochromatic 365-nm radiation (Tyrrell, R. M., and R. B. Webb, *Mutat. Res.* 19, 361; 1973, Webb, R. B., et al., *Radiat. Res.* 74, 298, 1978).

Data reported here suggest that error-prone and error-free components of DNA repair are differentially inhibited by broad spectrum near-UV radiation: in the repair-proficient strain WP2, BLB radiation selectively inhibits error-prone repair, requiring more of the damage to be processed by error-free components of excision repair, thereby decreasing the 254 nm induced mutant yield; however, in the excision-deficient strains, WP2s and WP6s, the remaining error-free components of repair are selectively inhibited, forcing more of the DNA lesions to be processed by the error-prone "SOS" component of postreplication repair, and increasing mutant yields (Figure 12.2, Part B). These data further suggest the possibility that a similar interaction of near-UV (320–400 nm) and mid-UV (290–320) wavelengths may reduce the mutagenic potential of full spectrum solar ultraviolet light on mammalian cells when compared to that expected with monochromatic radiation in the 290–320 nm wavelength range.

¹Sponsored by the Argonne Affirmative Action Program.

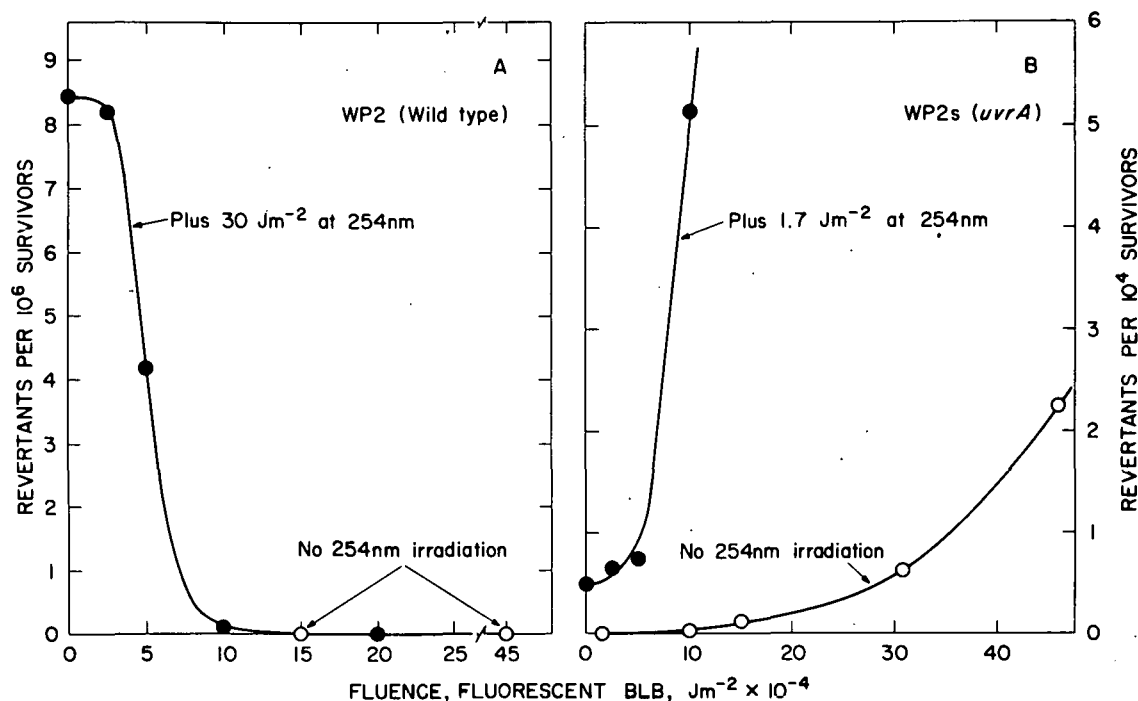


Fig. 12.2. The effect of prior fluorescent BLB (310-405 nm) irradiation on 254 nm-induced tryptophan revertant frequency in *E. coli* strains WP2 (wild type) (Panel A) and WP2s (*uvrA*) (Panel B). Mutant and total assays were on minimal agar plates supplemented with 2% nutrient broth. Fluence rates were approximately 65 W m^{-2} with BLB radiation and 0.5 W m^{-2} with 254-nm radiation.

NEAR-ULTRAVIOLET LETHALITY: NONRECIPROCAL SYNERGISTIC INTERACTION BETWEEN A NEAR-ULTRAVIOLET (365 nm) AND A VISIBLE (405 nm) WAVELENGTH IN *ESCHERICHIA COLI*

M. S. Brown and R. B. Webb

Near-UV radiation (320-400 nm) induces moderate sensitization of repair-proficient and single repair-deficient strains of *Escherichia coli* to X irradiation (Tyrrell, R. M., *Int. J. Radiat. Biol.* **25**, 373, 1974), mild (52°C) heat (Tyrrell, R. M., *Photochem. Photobiol.* **24**, 345, 1976), and 254-nm radiation (Webb, R. B., et al., *Radiat. Res.* **74**, 298, 1978). In addition, we have observed recently that a prior exposure to 365-nm radiation at fluences greater than $1 \times 10^6 \text{ J m}^{-2}$ enhances the lethal effects of 405-nm radiation on repair-proficient and *uvrA* strains of *E. coli* to a greater extent than do the other lethal agents that have been tested. Fluences of 365-nm radiation that yielded a surviving fraction of 0.1 (F_{10}) resulted in the following sensitization ratios (ratio of the F_{37} fluence at 405 nm

with a prior 365-nm exposure): Strain K12 AB1157 (wild type), 14 (Figure 12.3, Part A); Strain WP2 (wild type), 48; Strain WP2s (*uvrA*), 12; Strain WP2s (*uvrA thy*), 265. The maximum sensitization ratios obtained were: Strain K12 AB1157, 45; Strain WP2, 74; Strain WP2s, 48; Strain Wp2s (*thy*), 365. Very little sensitization to anoxic 405-nm radiation was observed, suggesting that 365-nm damage interacts with DNA lesions that are produced at 405 nm by an oxygen-dependent mechanism. DNA single-strand breaks (or alkali-labile bonds) were produced by $^{2}_{405}$ -nm radiation at 3.0×10^{-5} breaks per 2.5×10^9 daltons per J m^{-2} in the *polA* Strain P3478, while pyrimidine dimers were below detection.

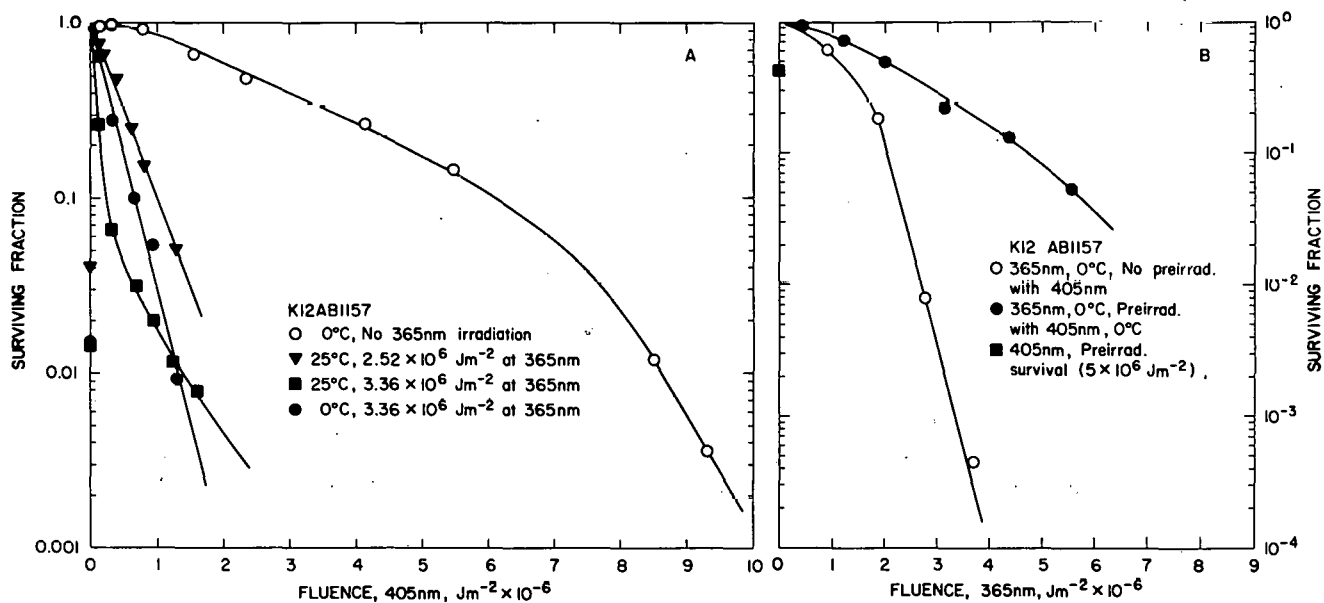


Fig. 12.3. Fluence-survival response of M9-buffer suspensions of stationary-phase cells of *E. coli* K12 AB1157 (wild type) at 405 nm (Part A) or 365 nm (Part B). Part A: Cell suspensions were irradiated at 405 nm with and without a prior irradiation at 365 nm. Part B: Cell suspensions were irradiated at 365 nm with and without a prior irradiation at 405 nm. Fluence rates were 600–1200 W m^{-2} at 365 nm and 400–650 W m^{-2} at 405 nm. Short wavelength stray light was reduced at 365 nm by a Corning 0-52 (1/2 thickness) absorption filter (1% transmittance 336 nm) and at 405 nm by an Optics Technology LP400 absorption filter (1% transmittance 370 nm).

In striking contrast to the strongly synergistic effect of 365-nm radiation on 405-nm lethality, a prior exposure to 405-nm light reduces the lethal effects at 365 nm in *E. coli* K12 AB1157 (Figure 12.3, Part B). This lack of reciprocity indicates that the 365–405 nm interaction is not a simple interference between two classes of DNA lesions. Furthermore, although inhibition of components of DNA repair by 365-nm irradiation has been demonstrated, such damage cannot account for the decrease in 365-nm lethality produced by prior

irradiation at 405 nm. However, if the uvrA endonuclease is selectively inhibited by 365-nm radiation, the presence of DNA single-strand breaks that are produced by 405-nm radiation might increase effective excision repair of 365-nm lesions. We propose that the strong sensitizing effect of 365-nm radiation on 405-nm lethality is the consequence of the strong inhibition of specific components of the DNA-repair systems that can remove or bypass 405-nm DNA lesions.

THE B_{s-1} CHEMOSTAT IN THE ABSENCE OF EXOGENOUS ACTIVATION AS A SENSITIVE INDICATOR OF MUTAGENICITY OF BENZO(A)PYRENE AND COMPLEX MIXTURES FROM HIGH-BTU GASIFICATION PLANT PROCESS STREAMS

B. S. Hass, R. B. Webb, and T. Gambill¹

We have developed chemostat cultures (Kubitschek, H. E., Introduction to Research with Continuous Cultures, Prentice Hall, Englewood Cliffs, NJ, 1970) of Escherichia coli B_{s-1} (lexA uvrB lon) as a new system for measuring bacterial mutagenicity. The system enables measurement of mutant frequency at low toxicant doses as a function of time and without metabolic activation. Benzo(a)pyrene (BaP) is a well-known carcinogen that is also mutagenic to bacteria in the presence of liver microsomal activation. Chemostat cultures of E. coli B_{s-1} were exposed to BaP, without any added activation enzyme, and the mutation rates to resistance to bacteriophage T5 were measured (Figure 12.4). A linear relationship as observed between BaP concentration and the mutation rate, expressed either as mutant frequency per day or mutant frequency per generation. The sensitivity of chemostat cultures of E. coli B_{s-1} to mutation induction by BaP without exogenous activation was about one third as great as that obtained with the Ames Salmonella assay system with optimal enzyme activation. With the chemostat cultures, BaP at a concentration of 0.2 μ M doubled the mutation rate observed without the mutagen (spontaneous). With the Ames technique, using Salmonella typhimurium TA98, BaP at 0.06 μ M produced a 2-fold increase in mutant yield over the spontaneous rate at optimal enzyme activation. Solubility of BaP required the presence of ethanol; however, ethanol at concentrations as high as 1% (v/v) did not alter mutation rate.

Several control experiments indicated no increase in mutagenesis. Benzo(e)pyrene, at most weakly mutagenic in Salmonella strains, was not mutagenic at a concentration of 5 M (Figure 12.4). Mutation rates in chemostat cultures of E. coli Strains B (wild type) or WP10 (recA) gave no mutational response to BaP.

A sample from the oil recirculation loop process stream of the HYGAS coal gasification plant was tested in the B_{s-1} chemostat system. The crude material was dissolved in dimethyl sulfoxide (Me₂SO) and added, with additional Me₂SO, to the culture medium to yield a final concentration of

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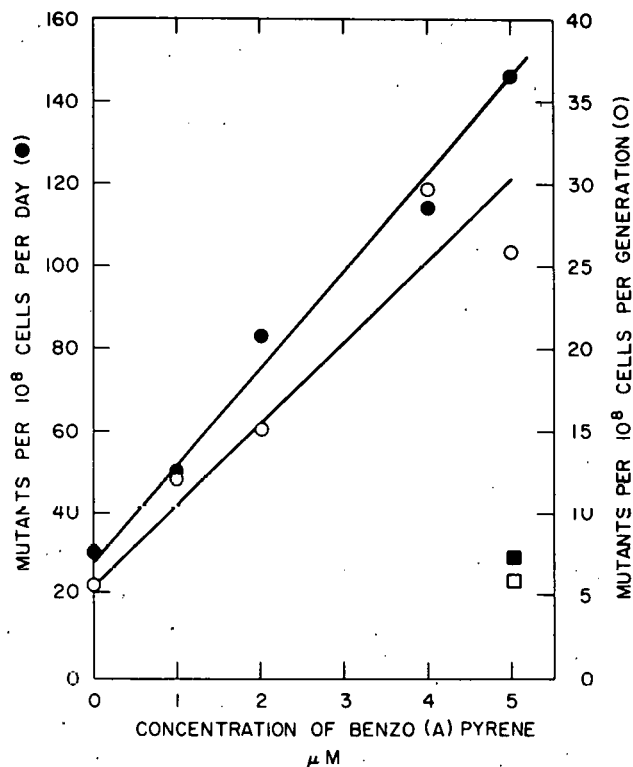


Fig. 12.4. Response of *E. coli* B_S-1 with a glucose-limited chemostat (50 mg/litre) to unactivated B(a)P as a function of chronological time (●) or of cell generation time (○). The symbols ■ and □ indicate the response of 5 μM BeP in terms of chronological and cell generation time, respectively.

approximately 80 μg/litre and an Me₂SO concentration of 0.05%. This low concentration of the crude oil sample mixture in the chemostat B_S-1 test system, without enzyme activation, induced a mutation rate of 55^{s-1} mutants per 10⁸ cells per day. It was thus at least as sensitive as the Ames *Salmonella* system with optimal enzyme activation.

A silica gel fraction from the nonvolatile residue of a process stream oil sample also was tested in the B_S-1 chemostat system; this fraction included phenols, carbonyls, and alcohols. The fraction was added to the culture medium at 1.26 μg/ml, comparable on a weight basis to the earlier concentration of 5 μM benzo(a)pyrene. The chemostat results are shown in Figure 12.5. The mutation rate from this fraction was 810 mutants per 10⁸ cells/day, corresponding to 643 mutants per 10⁸ cells/day/μg, which is about 25 times the spontaneous rate. In the Ames assay, with required activation, the same concentration increased the spontaneous colony number by slightly less than 2. Thus the chemostat assay, without enzyme activation, was about 20 times more sensitive than the Ames assay with activation.

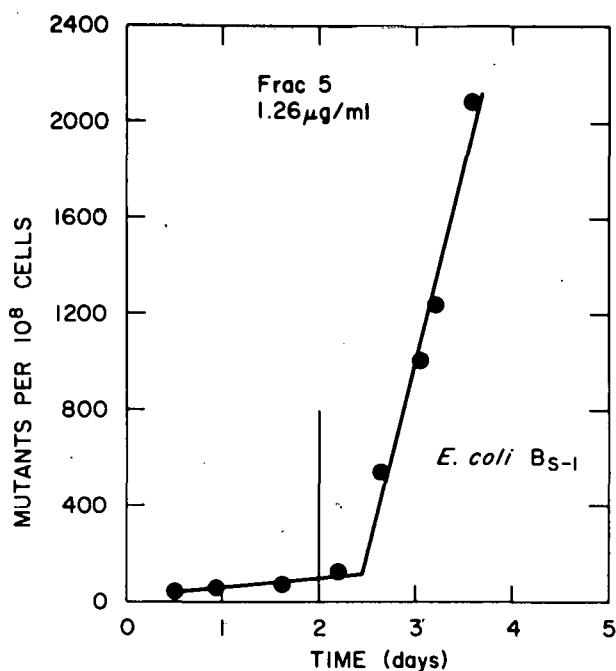


Fig. 12.5. Mutagenic response in the chemostat B_S-1 system exposed to a fraction from the HYGAS gasification oil recirculation stream eluted on silica gel with ethyl acetate.

SYNERGISTIC, ADDITIVE, AND ANTAGONISTIC MUTAGENIC EFFECTS OF TWO COMPONENT MIXTURES OF POLYCYCLIC AROMATIC HYDROCARBONS

B. S. Hass, E. Brooks,¹ K. Schumann,² and S. S. Dornfeld

The interaction of polycyclic aromatic hydrocarbon (PAH) carcinogens with homologous cocarcinogens and noncarcinogens was examined in the Ames *Salmonella* microsomal assay system. Binary mixtures of PAH's were prepared and tested with *Salmonella* Strain TA98 in the presence of S-9 rat liver microsomal activating enzymes. Results were considered synergistic when the two compounds together produced more mutants than the sum of the mutants produced individually, additive when the same number of mutants were produced, and antagonistic when fewer mutants were produced by the combined mutagens.

Results of representative examples, indexed by carcinogenic response (cocarcinogens, carcinogens, noncarcinogens), are shown in Table 12.3. The concentration ranges of the test substances were very low and did not exhibit any toxicity in the standard Ames test. The carcinogen benzo(a)pyrene (BaP) and the cocarcinogen benzo(e)pyrene interacted synergistically; in contrast, the cocarcinogen pyrene produced only an additive effect with BaP. Further, the noncarcinogen perylene interacted synergistically with BaP when added in

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²Spring 1979 participant in the Undergraduate Honors Research Participation Program, Skidmore College.

Table 12.3. Synergism and Antagonism between Mixed Mutagens in the Ames Test with Strain TA98 with Enzymatic Activation

Mixture	Concentration Range ($\mu\text{g}/\text{plate}$)	N	Ratio ^a \pm SE	Response
Benzo(a)pyrene (carcinogen) Benzo(e)pyrene (cocarcinogen)	0.25 -1.25	28	1.61 \pm 0.06	Synergism
Benzo(a)pyrene (carcinogen) Pyrene (cocarcinogen)	0.125-1.25	19	0.96 \pm 0.05	Additivity
Benzo(a)pyrene (carcinogen)	0.125-1.25	6	2.17 \pm 0.24	Synergism
Perylene (noncarcinogen)	0.625-2.5	15	0.63 \pm 0.05	Antagonism
Pyrene (cocarcinogen) Perylene (noncarcinogen)	0.625-2.5	3	0.36 \pm 0.08	Antagonism
1,2,3,4-dibenzanthracene (noncarcinogen) 1,2,5,6-dibenzanthracene (carcinogen)	0.25- 2.5	11	1.50 \pm 0.12	Synergism

^aRatio indicates the number of revertants in the binary mixture divided by the algebraic sum of the number of revertants of the individual components.

Me₂SO to give a concentration range 0.125 to 1.25 μg of perylene/plate, but had an antagonistic effect in the concentration range of 0.625 to 2.5 $\mu\text{g}/\text{plate}$. Perylene and pyrene also acted antagonistically.

Together, 1,2,5,6-dibenzanthracene, a strong carcinogen but a weak mutagen, and 1,2,3,4-dibenzanthracene, catalogued as a noncarcinogen but a strong mutagen, produced a synergistic response. In addition, observed responses depended upon strain; in Strain TA100, for example, benzo(a)pyrene and benzo(e)pyrene gave only an additive response.

In summary: (1) binary mixtures at very low, nontoxic concentrations of PAH's produce complex mutagenic responses that are not predicted by their carcinogenic potential; (2) mutagenic responses to the mixtures depend upon the concentrations of the components; and (3) the degree of interaction also depends upon the Salmonella strain used. These results indicate that the mutagenic response of a binary mixture is unlikely to represent the sum of responses of its individual components.

13. MAMMALIAN CELL BIOLOGYINTRODUCTION

M. M. Elkind

Damage and repair processes play a critical role in the production of altered cell properties by radiation and other environmental agents. In connection with mutation and transformation, for example, an altered phenotype cannot be expressed if the cell is killed. Hence, at the outset, damage-repair processes relative to cell survival need to be understood in order that similar appraisals may be made for other end points. The research of the Mammalian Cell Biology Group includes, therefore, a major emphasis on mechanisms of cell killing, first because of the relevance of lethality to the expression of other changes, and second because the cellular and molecular biology of nonlethal changes may have elements in common with those of lethality. To this end, we have studied damage and repair after ionizing and nonionizing radiations, and their combination, in respect to cell killing and neoplastic transformation. We have also further pursued the photodynamic properties of polycyclic aromatic hydrocarbons.

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REPAIR OF POTENTIALLY LETHAL DAMAGE AND "SINGLE-HIT" KILLING

H. Utsumi, P. Ross-Riveros, C. M. Liu, and M. M. Elkind

Actively growing V79 Chinese hamster cells treated with anisotonic phosphate buffered saline (PBS) after X-irradiation show lower survival levels than cells treated with isotonic PBS or cells promptly incubated with complete medium immediately after irradiation. The anisotonic PBS solutions used were not toxic in unirradiated cells. The sensitization of irradiated cells results from both hypotonic as well as hypertonic NaCl concentrations in PBS, is strongly dependent on both temperature and time, and is expressed mainly as an increase in the final slope of the single-dose survival curve. Cells at essentially all ages in the division cycle are sensitized. The oxygen enhancement ratios of cells receiving an anisotonic posttreatment are almost equal, suggesting that typical radiochemically induced lesions, rather than nonspecific stress, are responsible for the enhanced lethality. After two X-ray dose fractions, the response of cells sensitized after both fractions by anisotonic posttreatment is similar to that obtained for cells treated isotonicly after the two fractions and indicates that sublethal damage repair is not influenced by the enhanced expression of potentially lethal damage. Independence of potentially lethal damage repair from sublethal damage repair is further suggested by the more rapid rate of the former compared to the latter. In contrast to the modification of expression of potentially lethal damage in plateau phase cells following lethal exposures to nonionizing radiations, with or without the prior incorporation of 5-bromodeoxyuridine into DNA, essentially no additional damage is expressed after the anisotonic PBS treatment of actively growing cells. Thus, essential differences in the molecular bases of cell killing by nonionizing versus ionizing radiation are suggested. The proposal is advanced that enhanced expression of potentially lethal damage after X-irradiation results from a destabilization of the structural relationship between DNA and the nuclear envelope, or the nuclear envelope and the nuclear protein matrix, as a consequence of osmotic changes produced by anisotonic treatment.

In addition to demonstrating repair of potentially lethal damage after mid-to-large ionizing radiation doses in repair-competent cells, the technique of postirradiation treatment with anisotonic PBS solutions also yielded information on the repair of such damage after small doses. It is generally acknowledged that the surviving fraction S of mammalian cells may be described by

$$S = e^{-\alpha D} [M(D)],$$

where α is a constant and $M(D)$ is a single-valued function of the dose D to signify a multievent, damage accumulation mode of lethality. Using fission-spectrum neutrons as well as X-rays, we found that enhanced cell killing after small doses could be effected by hypertonic PBS. These data indicate that Chinese hamster cells ordinarily effect the repair of potentially lethal damage throughout the measurable survival range and, in particular, that so-called "single-hit" killing can be subject to modification by cellular repair.

CHANGE IN REPAIR COMPETENCY AFTER 5-BROMODEOXYURIDINE PULSE LABELING AND NEAR-ULTRAVIOLET LIGHT

M. P. Hagan¹ and M. M. Elkind

To advance the understanding of the role of DNA damage in cell killing, it is useful to use treatments in which one or another form of DNA lesion(s) is produced with high frequency. Such is the case in respect to the light sensitivity that results when 5-bromodeoxyuridine (BUdR) replaces thymidine in the genome of a cell. For this reason, studies have been pursued of the mechanism of BUdR photolethality.

Synchronized V79 Chinese hamster cells, pulse-labeled with 5-bromodeoxyuridine, show marked changes in the sensitivity to near-ultraviolet light during the cell cycle. Cells are least sensitive during the remainder of the S-phase following the BUdR pulse. They become maximally sensitive in the next cell cycle when the BUdR-labeled DNA presumably serves as the template for replication. A return to relative insensitivity follows during the remainder of that S-phase. When BUdR is given both near the beginning and again near the end of the same S-phase, the increase in survival does not occur until DNA synthesis progresses beyond the time when the DNA made during the second pulse serves as a template. Furthermore, cells in the resistant phases of the cell cycle are sensitized by 1-2 mM caffeine. The data support the hypothesis that lesions in the DNA containing BUdR are effectively repaired after semiconservative replication. The data also indicate that saturation of repair capacity and not target multiplicity is responsible for the appearance of a shoulder on the survival curves.

DAMAGE-REPAIR STUDIES OF THE NEOPLASTIC TRANSFORMATION OF MOUSE C3H/10T1/2 CELLS USING FRACTIONATED DOSES OF X-RAYS AND FISSION-SPECTRUM NEUTRONS

A. Han, C. Hill,² J. L. Dainko, E. M. Buess, and M. M. Elkind

It is well known that dose fractionation results in increased survival of mammalian cells because of the repair of sublethal damage during the inter-fraction interval. We have initiated a study of the influence of dose fractionation on neoplastic transformation to determine whether "subtransformation" damage is also repaired.

A mouse embryo-derived cell line, C3H/10T1/2, was used to measure the frequency of in vitro neoplastic transformation induced by 50-kVP X-rays and by fission-spectrum neutrons from the JANUS reactor in the Division. The transformation frequency after single X-ray doses rises exponentially, reaching a plateau at about 3×10^{-3} transformant/survivor. The induction

¹Laboratory Graduate Program Participant, University of Illinois, Urbana.

²Resident Associate, Division of Biological and Medical Research.

curve following single neutron doses, although qualitatively similar, initially rises more steeply and levels off at a maximum of about 6×10^{-3} transformant/survivor. For both radiations, transformation frequency varies with changes in the number of viable cells per dish, showing about a 10-fold decrease in transformation frequency when the number of viable cells per 90-mm dish was increased from about 300 to 1000. Fractionation of a total X-ray dose of 700 rads into two fractions separated by various time intervals from 2 to 16 hours results in an approximately 6-fold increase in survival and reduction in transformation frequency. Fractionation of the total neutron dose of 378 rads by various time intervals from 3 to 24 hours has no effect upon cell survival, and transformation frequency declines by a factor of only about 1.7 at most. Cells derived from transformed foci formed fibrosarcomas when injected into appropriately treated mice.

THE INTERACTION OF DAMAGE DUE TO "SUNLIGHT" AND X-RAYS

A. Han, J. L. Dainko, C. M. Liu, and M. M. Elkind

We are all exposed to the radiations from sunlight during most of our lifetimes. Since the incidences of basal and squamous cell skin cancer have increased appreciably during the past several decades, it is important to determine whether damage resulting from sunlight interacts with damage from other agents. To this end, a study was made of the combined lethal actions of a sunlight-simulating source of near-UV light and X-rays.

Enhanced cell killing due to the combined exposure of "sunlight" (Westinghouse Sun Lamps, FS20) and X-rays, indicative of damage interaction, is observed at all stages of the cell cycle. The greatest interaction is observed in the middle of the DNA synthetic phase. At equal survival levels, "sunlight" damage is only partly additive to X-ray damage and vice versa. Loss of damage interaction between radiations is more rapid after a first dose of X-rays than after a first dose of sunlight. The data indicate that the damage due to near-UV and X-rays interacts and is partly additive.

A second study of the combined action of possible environmental agents and sunlight concerns polycyclic aromatic hydrocarbons (PAH's). A number of PAH's are coal derived and are known to be carcinogens. With the prospect of our increasing reliance on coal as a fuel for power production, we have started a study of the influence that certain photochemically active PAH's have on cell killing caused by a filtered and unfiltered source of the sunlight-simulating near-UV light.

PHOTODYNAMIC CYTOTOXICITY IN MAMMALIAN CELLS EXPOSED TO SUNLIGHT-SIMULATING
NEAR-ULTRAVIOLET LIGHT IN THE PRESENCE OF THE CARCINOGEN
7,12-DIMETHYLBENZ(A)ANTHRACENE

H. Utsumi, G. R. Lankas, J. L. Dainko, and M. M. Elkind

The coal-derived carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) added to cultures of V79 Chinese hamster, mouse C3H/10T1/2, and human HeLa cells enhances photolethality induced by the sunlight-simulating emission from Westinghouse Sun Lamps (FS20, \sim 290-400 nm). The enhancement occurs only in the presence of oxygen and only when the DMBA precedes the irradiation; treatment of cells with DMBA after irradiation is without lethal effects. The endoperoxide of DMBA is ineffective both before as well as after irradiation, and DMBA incubation before far-UV exposure (254 nm) is protective. Cells rendered photosensitive by incubation with DMBA rapidly lose their sensitivity (in less than 10 minutes at 37°C) if incubated in a DMBA-free solution containing serum, but maintain their sensitivity at least for several hours if a serum-free solution is used. Although DMBA enhances light-induced killing of cells in all phases of the cycle, those undergoing DNA synthesis are sensitized the most. The data support photodynamic lethality due to one or both of the following: (1) the reaction with DNA of either DMBA radicals followed by oxidation, or DMBA-produced single oxygen; or (2) the peroxidation of lysosomal membranes followed by the release of hydrolases, include DNases.

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14. CARCINOGENESISINTRODUCTION

Christopher A. Reilly, Jr.

Many of the chemical, physical, and biological agents known to cause cancer are now present in our environment. In addition, there are numerous other agents that, although not carcinogenic themselves, increase tumor incidence by interacting with carcinogens. The possibility of such interactions must be taken into consideration for proper assessment of the human cancer risk. Thus, to evaluate accurately the effects of environmental contamination with cancer-causing materials from the existing and emerging energy technologies, it is necessary to understand the nature and underlying mechanisms of carcinogen/cocarcinogen interactions. The general objective of the Carcinogenesis Program is to examine these mechanisms. At the same time we are also attempting to develop rapid and reliable methods for assessing the tumorigenic risk from potential carcinogenic and cocarcinogenic energy-related effluents. These efforts complement and extend ongoing Divisional toxicology studies in coal, radiation, and energy storage and transmission.

Particularly noteworthy accomplishments during the year include the following. (1) Further insight into the role of phenobarbital as a promoter of hepatic tumors induced by a chemical carcinogen was achieved with the demonstration that the observed effect is due to the appearance of new tumors, not to the accelerated growth of tumors already present. (2) The recent adaptation of liver cells to growth in an in vitro culture system will greatly facilitate studies on the regulation of liver enzymes. (3) The question of whether retroviruses are involved in the induction of tumors by radiation has come closer to an answer with the demonstration that most, if not all, bone cancers induced by ⁹⁰Sr in CF#1 and X/Gf mice contain such viruses. Animal tests suggest that some of these viruses can cause sarcomas. (4) Significant progress has been made in developing several systems for early detection of carcinogenic and cocarcinogenic agents. These include the demonstration that rat livers are responsive to polycyclic aromatic hydrocarbons and that there are new chromosomal proteins in hepatic tumors that can be detected immunologically.

The following report is organized into two sections, the first dealing with mechanisms of cocarcinogenesis and the second with the development of test systems.

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MECHANISMS OF COCARCINOGENESIS

This section includes the description of experiments on the role of gene regulation in rat hepatic neoplasia, on tumor promotion, on the interaction of viruses and radiation in the induction of murine bone tumors, and on modulating factors in skin carcinogenesis.

CIRCADIAN REGULATION OF ORNITHINE AMINOTRANSFERASE AND SERINE DEHYDRATASE SYNTHESIS IN RAT LIVER

K. B. Ekelman, C. Peraino, A. M. Prapuolenis, and D. P. Peterson¹

Studies on rat liver enzyme regulation have involved the comparison of the responses of two amino acid catabolizing enzymes, ornithine aminotransferase and serine dehydratase, following exposure of rats to dietary and hormonal stimuli. Previous investigations in this laboratory have demonstrated that circadian oscillations in the synthesis of the two enzymes persist in rats maintained on a high protein diet, and that the phase of the synthesis cycles determines the response of the enzyme regulatory system to hormonal stimuli.

A series of experiments are now in progress that will enable us to determine which of several possible stimuli (i.e., dietary protein, light, and steroid availability) are responsible for the maintenance and phasing of circadian enzyme synthesis in rat liver. The results of an investigation designed to determine the effects of a 12-hour out-of-phase meal schedule on ornithine aminotransferase and serine dehydratase synthesis cycles suggest that this meal shift altered the phasing of the ornithine aminotransferase synthesis cycle and eliminated circadian fluctuations in serine dehydratase synthesis; these responses do not appear to depend on differences in the amount of food consumed by rats during the restricted meal feedings.

Stomach emptying experiments performed on rats maintained on 2-hour restricted meals suggest that rats fed at or near the commencement of the light phase do not adapt metabolically to a restricted meal as well as do rats fed at or near the commencement of the dark phase of the light/dark cycle.

Reports of the persistence of cyclic liver enzyme levels in cultures of isolated rat hepatocytes have suggested that primary control of these enzyme fluctuations is regulated within the cell and not by the action of periodic extracellular stimuli *in vivo*. For this reason the *in vivo* regulation studies have been accompanied by *in vitro* experiments on rat hepatocytes maintained in primary culture. A sensitive high pressure liquid chromatographic assay for the presence of ornithine aminotransferase has been developed for the detection of enzyme levels in cultured hepatocytes.

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A comparison of ornithine aminotransferase regulation in primary hepatocyte cultures and in the H-35 hepatoma cell line is presently being undertaken, and an attempt will be made to relate specific observed alterations to the transformed character of the cell line.

DIETARY PHENOBARBITAL EFFECTS ON FOOD UTILIZATION EFFICIENCY AND CIRCADIAN CYCLING OF FOOD INTAKE AND DEEP-BODY TEMPERATURE IN RATS

C. Peraino, C. F. Ehret,¹ K. R. Groh,¹ J. C. Meinert,¹
G. D'Arcy-Gomez,² and A. M. Prapuolenis

In recent experiments comparing tumorigenic enhancing effects of phenobarbital at various dietary concentrations, we observed that 0.25% dietary phenobarbital reduced the growth rate of the rats and produced noticeable somnolence between approximately 11:00 a.m. and 3:00 p.m. daily. The most obvious interpretation of these observations is that the behavioral changes induced in the rat by the high dietary phenobarbital probably caused a reduction in food intake with a consequent slowing of growth. This interpretation required verification, however, since it was necessary to determine whether our tumorigenesis studies would be complicated by phenobarbital-mediated changes in the level of food intake. The present study, therefore, analyzed the effects of 0.25% dietary phenobarbital on growth rates and food intake levels in rats. In addition, in view of the cyclic behavioral changes noted above, phenobarbital effects on the circadian fluctuations in deep-body temperature and food intake were measured by means of an automated monitoring system.

The results showed that the daily food intake was similar in untreated control rats and those receiving 0.25% dietary phenobarbital, but the efficiency of food utilization for growth was 20% lower in the phenobarbital-treated rats. Further, the amplitude of the circadian oscillation of deep body temperature in the rats fed phenobarbital was twice that in the controls and the attainment of peak body temperatures was advanced by approximately 2 hours. A comparison of food intake patterns showed that rats fed the control diet eat around the clock, albeit at a greater rate during dark than during light phase, whereas rats on phenobarbital start to eat earlier in the dark phase and confine their feeding almost exclusively to early dark phase. These results suggest that high dietary phenobarbital produces hitherto unsuspected metabolic regulatory and nutritional effects. Certain of these effects may influence susceptibility to tumorigenesis in the liver. This possibility is supported by our recent observation that 0.25% dietary phenobarbital enhanced tumorigenesis more than lower doses, but only after a prolonged lag interval during which tumorigenesis was lower than that with the lower phenobarbital doses.

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EFFECTS OF VARYING THE DIETARY CONCENTRATION OF PHENOBARBITAL ON ENHANCEMENT OF 2-ACETYLAMINOFLUORENE-INDUCED HEPATIC TUMORIGENESIS

C. Peraino, E. F. Staffeldt, V. A. Ludeman, K. A. Rettman,
D. A. Haugen,¹ and R. J. M. Fry²

In prior studies we demonstrated that the short-term exposure of rats to 2-acetylaminofluorene (AAF), a liver carcinogen (tumor initiator), produced a low incidence of liver tumors. When the AAF treatment was followed by the feeding of a phenobarbital-supplemented diet the incidence of these tumors was markedly increased. Several experiments have been conducted to define the characteristics of the tumorigenic enhancing effect of phenobarbital in an effort to gain insight into mechanisms underlying the postinitiation stage of tumorigenesis. The present investigation was designed to compare the tumorigenic enhancing effect of four different dietary concentrations of phenobarbital, 0.002%, 0.01%, 0.05%, and 0.25%. This study has just been concluded and evaluation of the data is not yet complete, but the following preliminary interpretations are possible.

1) Although the onset of tumorigenesis was delayed in comparison with that in previous experiments, the final tumor incidence level resembled that in previous experiments. This suggests that the time to first appearance of tumors and level of tumor incidence are not necessarily linked.

2) A computer-based statistical comparison of the tumor incidence kinetics for the 0%, 0.01%, and 0.05% phenobarbital treatment groups indicates that the times to first tumor appearance and the attainment of plateau levels of tumor incidence were similar among the three groups although the plateau levels increased with increasing phenobarbital dosage. These data suggest that phenobarbital increases tumor incidence without accelerating tumor growth.

3) A dietary phenobarbital concentration of 0.25% had the greatest tumorigenic enhancing effect, but only after a prolonged lag interval during which tumorigenesis was lower than with the lower phenobarbital doses. The lag suggests that the high phenobarbital dosage produced metabolic changes that temporarily interfered with the enhancement phenomenon.

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EFFECTS OF BUTYLATED HYDROXYTOLUENE ON HEPATIC MICROSOMAL CYTOCHROME P-450

D. A. Haugen¹ and J. P. Hardwick²

Cytochrome P-450 is a hemoprotein that is part of the microsomal monooxygenase system that metabolizes carcinogens, drugs, and environmental chemicals. The concentration of hepatic cytochrome P-450 can be increased by administration of a variety of drugs including phenobarbital, a promoter of liver hepatic tumors (Peraino, C., et al., Cancer Res. 31, 1506, 1971). The present study compares the biochemical effects of two promoters, phenobarbital and the food preservative BHT (butylated hydroxytoluene), on the level of microsomal cytochrome P-450.

The concentration of cytochrome P-450 can be measured spectrophotometrically due to the presence of the chromophoric heme moiety. In rats receiving dietary phenobarbital, the amount of cytochrome P-450 increased in a dose-dependent manner, with stable levels achieved after 5 to 7 days. Dietary administration of BHT caused only a transient increase in the concentration of cytochrome P-450 so that after 3 to 4 days no effect was detected despite continued administration.

The cytochrome P-450 can also be detected electrophoretically, but in this method the heme is dissociated from the protein so that only the apoprotein is observed. Administration of either phenobarbital or BHT caused a characteristic dose-dependent increase in the levels of several microsomal proteins, including the apoprotein(s) of cytochrome P-450. The pattern of these changes was indistinguishable for the two treatments. Thus, although BHT caused an increase in the level of the apoprotein(s) of cytochrome P-450, it did not cause an increase in the level of the complete hemoprotein, as detected spectrophotometrically.

Experiments were designed to explore the possibility that BHT interferes with heme metabolism at the level of heme synthesis, degradation, or its incorporation into the apoprotein. Administration of BHT caused an increase in both heme synthesis and degradation, as indicated by increased levels of δ -aminolevulinate synthetase and heme oxygenase, respectively. The level of free heme was not altered by BHT treatment. Addition of heme to microsomes from BHT-treated animals increased the concentration of cytochrome P-450 as detected spectrophotometrically, suggesting that heme had combined with apocytochrome P-450. Simultaneous administration of BHT and phenobarbital did not decrease the effectiveness of phenobarbital in its induction of cytochrome P-450.

Although the effect of BHT is obviously complex, and not yet well understood, the present results suggest that it causes induction of the apoprotein of cytochrome P-450, but interferes with the incorporation of the heme which is required for the formation of the complete cytochrome.

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TWO-DIMENSIONAL ELECTROPHORETIC ANALYSIS OF RAT LIVER AND NOVIKOFF HEPATOMA PROTEINS

K. E. Willard,¹ T. E. O'Connor, and N. G. Anderson²

Proteins from rat liver and Novikoff hepatoma cells have been mapped by isoelectric focusing in the first dimension and sodium dodecyl sulfate electrophoresis in the second, using the ISO-DALT system developed in the Molecular Anatomy Program (Section 17). However, this conventional isoelectric focusing did not give satisfactory resolution of the more basic proteins, which appeared as streaks. The BASO technique was designed to obtain complete resolution of these basic proteins in the first dimension. In this technique, samples were solubilized in a mixture of phosphatidyl choline and urea, loaded at the acid end of the gel, and electrophoresed for 3200 to 4000 volt-hours instead of the 10,000 volt-hours used in isoelectric focusing. Use of this technique followed by sodium dodecyl sulfate electrophoresis (i.e., the BASO-DALT system) enabled identification of the spots corresponding to the five histones (Figure 14.1A).

Considerable differences in the protein patterns of rat liver and Novikoff hepatoma have been detected in both the cytosol and nuclear fractions (Figure 14.1). The proteins found to be common among the patterns are the major cellular proteins which are found in most tissues. Therefore, it is concluded that Novikoff hepatoma cells are greatly different from normal liver and suggested that differences could be due to malignant transformation and/or to changes that have occurred because of long-term transfer of the tumor in the ascitic form.

PHOTOCHEMICAL INDUCTION OF SKIN TUMORS

D. D. Grube, K. A. Rettman, and R. J. M. Fry³

Certain of the furocoumarins such as 8-methoxypsoralen (8-MOP) are chemical sensitizers that photoreact with nucleic acids when exposed to long wavelength ultraviolet light (320-400 nm), extending the ultraviolet light (UV) action spectrum for lethal, mutagenic, and carcinogenic effects. By examining the UV dose-response relationship with or without chemical photosensitizers as a function of molecular lesions (psoralen bifunctional adducts--interstrand cross-links), acute cutaneous responses, and the initiation and expression of tumors in the skin of hairless mice, it should be possible to identify and quantitate the wavelength spectra and specific adducts involved in photocarcinogenesis. In addition, by using a chemical promoter, it is possible to determine whether exposure requirements differ between initiation and expression of tumors.

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²Molecular Anatomy Program.

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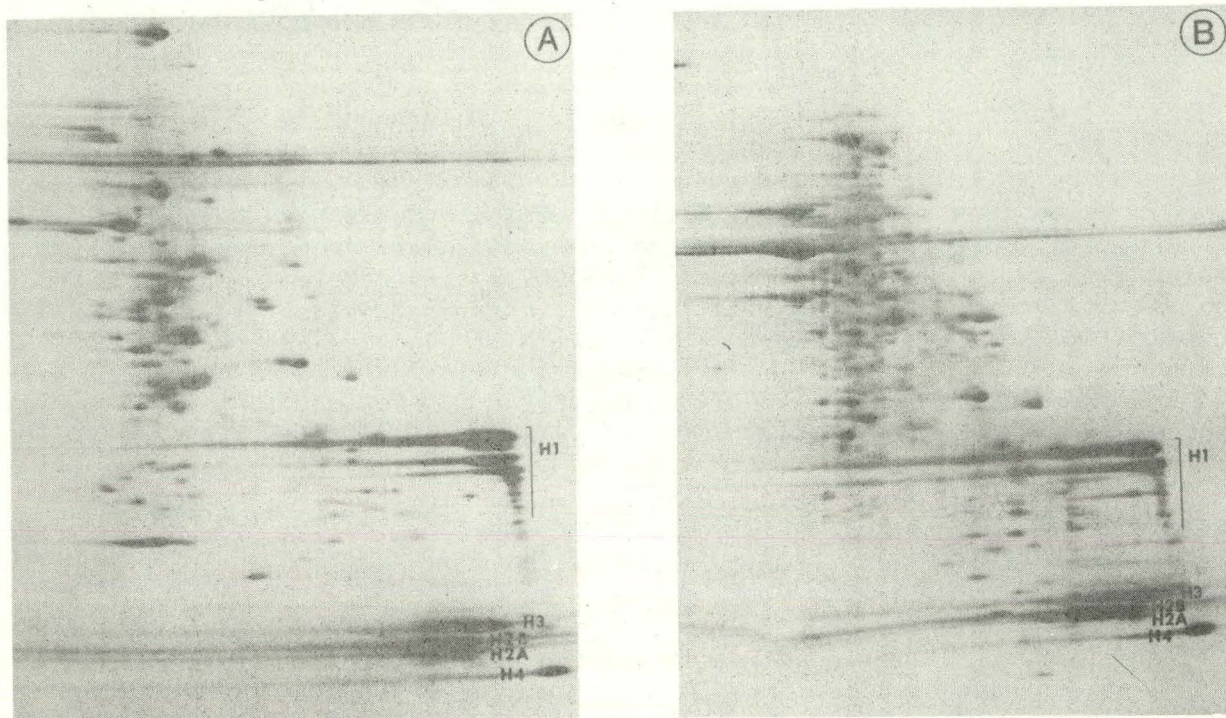


Fig. 14.1. BASO-DALT patterns of isolated nuclei from rat liver cells and Novikoff hepatoma cells. The first dimension gels were electrophoresed for 3200 volt-hours using LKB 3.5-10 ampholines in the ISO-DALT apparatus. The gels are oriented with the basic end to the right and the acidic end to the left. (A) BASO-DALT pattern of rat liver nuclei. (B) BASO-DALT pattern of Novikoff hepatoma nuclei. The histones H1, H2A, H2B, H3, and H4 are identified.

The induction of psoralen adducts in epidermal DNA and cytokinetic responses were used to (1) define fluence adjustments for inducing biologically equivalent damage with 8-MOP compound and different spectra of UV, and (2) determine the limits of exposure that induced stabilized cutaneous damage from which cumulative doses could be estimated. The preliminary results in Figure 14.2 show an increase in percent of mice with malignant skin tumors as a function of cumulative UV irradiation. Within a defined but tumorigenic

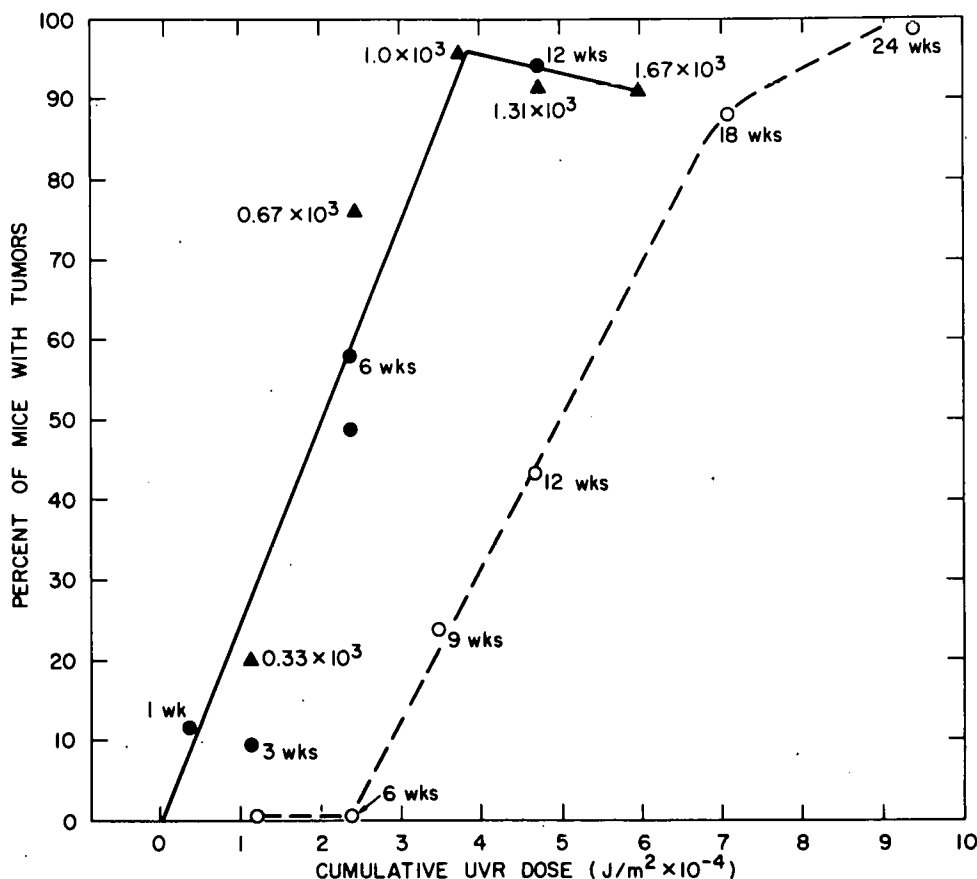


Fig. 14.2. Cumulative percent of SKH:hairless-1 female mice with one or more epidermal carcinomas induced by fractionated exposures, 3x/wk for the number of weeks noted, to combined treatments of 250 μg 8-MOP (topically) followed 45 minutes later by either (1) $1.31 \times 10^3 \text{ J m}^{-2}$, 320-400 nm UV radiation (\bullet, \circ), or (2) exposures 3x/wk for 12 weeks to 0.33, 0.67, 1.0, 1.31, or $1.67 \times 10^3 \text{ J m}^{-2}$ 320-400 nm UV radiation (\blacktriangle). In selected exposure regimens (\bullet, \blacktriangle), groups of mice additionally received topical applications of 5 μg TPA, 3x/wk for 52 weeks; this treatment was initiated one week after the termination of 8-MOP plus UV treatments.

range of fractionated UV exposures ($0.33 \times 10^3 \text{ J m}^{-2}$ to $1.31 \times 10^3 \text{ J m}^{-2}$), the number of psoralen adducts per cell (data not shown) and the number of epidermal carcinomas increased proportionately as a function of the cumulative radiation dose. Exposure to only UV radiation or 8-MOP, alone, or either treatment followed by promotion with 12-O-tetradecanoyl-phorbol-13-acetate (TPA) was ineffective in the induction of tumors. Further, with fractionations of 3x/wk, equal total doses differing only in the fluence per fraction or interval of exposure appear to be equally effective in the induction of tumors when animals were additionally treated with TPA. As shown in Figure 14.2, promotion with TPA effectively reduced the UV dose requirements for the induction of skin tumors, and the results suggest that the later exposures in the nonpromoted groups of mice are associated with both initiation and expression events. The promotion experiments using TPA show that photocarcinogenesis, as chemical carcinogenesis, is a multistage process and the data strongly implicate psoralen adducts as the lesion underlying initiation events. Recently completed experiments with the furocoumarin 4,5'8-trimethoxypsoralen support these observations.

BIOCHEMICAL AND BIOPHYSICAL CHARACTERIZATION OF FBJ AND FBR MURINE OSTEOSARCOMA VIRUSES

E. W. Chan, P. J. Dale, I. L. Greco, C. K. Lee, and K. Rupprecht¹

The fundamental properties of two earlier murine osteosarcoma viral isolates, FBJ (Finkel, M. P., et al., *Science* 151, 698, 1966) and FBR (Finkel, M. P., et al., Proceedings of the Twenty-fourth Symposium of the Colston Research Society, Vol. 24 of the Colston Papers, Butterworths Scientific Publications, London, 1973, p. 353) have been determined. In sucrose, they band at 1.16 g/ml. Their genomes consist of a 70S RNA that dissociates readily on heating into 34-35S subunits. They possess a reverse transcriptase that shows a preference for Mn^{+2} over Mg^{+2} . The enzyme is capable of catalyzing the synthesis of faithful and specific ³H-DNA probes that are complementary to their associated viral RNA genome.

Molecular hybridization studies with such probes show that the two viruses are genomically very closely related to each other but are totally unrelated to other nonmurine Type C viruses of chicken, cat, baboon, woolly monkey, and gibbon ape origin. Extensive reciprocal hybridization data between FBJ and FBR and other murine Type C viruses indicate they form a group distinguishable from the five previously established murine viral subgroups. They are very closely related to the AKR, and BALB/c endogenous viruses but are only partially related to the NIH Swiss endogenous, Rauscher, and Moloney subgroup viruses.

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These data indicate FBJ and FBR viruses have the biochemical and biophysical properties of Type C murine retroviruses, of which they constitute a distinctly identifiable subgroup.

IMMUNOLOGICAL CHARACTERIZATION OF MURINE OSTEOSARCOMA VIRUSES FBJ AND FBR

L. Bodoni, J. R. Mitchen, B. Amsler,¹ E. W. Chan, M. P. Finkel, and C. A. Reilly, Jr.

We are currently determining the immunological properties of FBJ virus (Finkel, M. P., et al., *Science* 151, 698, 1966) and FBR virus (Finkel, M. P., et al., Proceedings of the Twenty-fourth Symposium of the Colston Research Society, Vol. 24 of the Colston Papers, Butterworths Scientific Publications, London, 1973, p. 353). Fractionation of the viral proteins of FBJ and FBR by polyacrylamide gel electrophoresis resulted in electrophoretic patterns similar to those of other murine retroviruses. Apart from the large envelope proteins gp^{69/71}, the major p30, and the low molecular weight p10, FBJ and FBR have a p11 protein that is apparently a unique size class. Both viruses lack a p15 protein.

Rabbit anti-FBJ and anti-FBR sera were cross-reactive with the p30 proteins of FBJ, FBR, and two strains of murine leukemia viruses, Rauscher and AKR, in a double gel immunodiffusion assay. The precipitin lines of identity indicated that FBJ and FBR viruses have the group-specific antigen of murine retroviruses.

Sensitive competitive radioimmunoassays have been developed for the p30 proteins of FBJ and FBR viruses. Viral proteins were prelabeled to a high specific activity with ¹²⁵I by an enzyme-bead procedure, fractionated by slab gel electrophoresis, and isolated quantitatively by elution from appropriate slices of the gel. Competitive radioimmunoassays were set up using limiting quantities of ¹²⁵I-p30 and pretitrated antisera. Such assays could detect nanogram quantities of unlabeled homologous viral protein. Competition by other murine Type C viruses, such as Rauscher and AKR, gave profiles and slopes identical to those for FBJ and FBR. These data are consistent with those from the immunodiffusion studies. Similar competitive radioimmunoassays that should contain unique antigenic determinants are being developed for the p11 and p10 proteins. These should provide assays for specific detection of FBJ and/or FBR viruses.

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IN VITRO PROPERTIES OF A BENIGN MURINE BONE TUMOR VIRUS

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M. P. Finkel, and C. A. Reilly, Jr.

A virus isolated in our laboratory, RFB, has the unique property of inducing osteomas. This benign bone tumor virus came from the same CF#1 mouse strain from which the malignant bone tumor virus FBJ was isolated. We are now comparing this most interesting pair of viruses, benign and malignant, especially with respect to transformation.

RFB and FBJ showed similar host range specificities in cell culture. Both infect and replicate to high titers in whole embryonic cell cultures derived from CF#1, CBA, and NIH Swiss mice, but not from X/Gf mice. Like FBJ, RFB virus infects only murine cell lines and is not infectious for cell lines of hamster, rabbit, cat, mink, dog, sheep, cow, monkey, and human origin. Various murine cell lines are being tested for suitability for mass production of the benign bone tumor virus. Preliminary testing showed that RFB-infected cells are capable of growth in soft agar, an in vitro indicator of cell transformation. However, unlike FBJ, RFB virus failed to induce morphologic transformation in cultures from susceptible hosts, although both readily induced XC plaques. Therefore, attempts are being made to develop a colony transformation assay in soft agar for this benign virus.

ISOLATION AND CHARACTERIZATION OF RETROVIRUSES FROM ⁹⁰Sr-INDUCED OSTEOSARCOMAS

M. P. Finkel, C. K. Lee, E. W. Chan, L. S. Lombard, G. Rockus, V. A. Pahnke, I. L. Greco, P. J. Dale, M. F. Williams, and C. A. Reilly, Jr.

The hypothesis that radiation causes cancer by activating endogenous viral information is being tested in a system using ⁹⁰Sr to induce bone cancer in CF#1 and X/Gf mice. From each of these strains we have previously isolated osteosarcoma viruses that are being used to develop specific probes and antibodies to monitor virus expression during ⁹⁰Sr-induction of bone cancer (see preceding reports).

Current data indicate that probably all ⁹⁰Sr-induced bone sarcomas produce RNA virus, primarily of the helper or associated type. The experimental approaches have been: (1) to propagate the bone tumors in cell culture either directly or after cloning in soft agar; (2) to infect susceptible mouse cell lines, Sc-1 or D55, with tumor extracts; and (3) to cocultivate tumor

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cells with Sc-1 or D55 cells. Spent culture media from these cells are then tested for progeny virus by assaying for reverse transcriptase (the specific enzyme of retroviruses) and the ability to produce plaques on XC cells (an indicator of helper or associated virus) or foci of transformation in cultures of appropriate cells (an indicator of sarcoma virus).

As shown in Table 14.1 reverse transcriptase and the XC plaque assay have been positive in almost all cases; the focus assay has been negative. Further testing in mice, however, indicates the presence of sarcoma activity in CF#1 bone tumors and leukemia activities in X/Gf bone tumors. The *in vivo* test is done by injecting the spent tissue culture medium into newborn mice, which are then watched for tumor development. In the CF#1 strain there has been a marked increase over normal in the number of sarcomas (Figure 14.3). To date there have been 15 osteosarcomas, 5 fibrosarcomas, 1 liposarcoma, 1 leiomyosarcoma, 1 malignant hemangiopericytoma, and 1 neurofibrosarcoma. In the X/Gf strain the increase has been in the number of tumors of the reticular tissues; the incidence in one group is now 70%, with 6 of 33 mice still alive. It is important to note here that in the initial isolation of the X/Gf osteosarcoma virus from ⁹⁰Sr-induced osteogenic sarcomas, tumors of the reticular tissues, not osteosarcomas, appeared in the first and second cell-free passages.

Table 14.1. *In Vitro* Tests for Presence of Retroviruses (number of positive tests/total number of tests).

	Strain	Reverse Transcriptase	XC Plaque Assay	Focus-forming Assay
Direct ^a	CF#1	21/25	5/5	0/6
	X/Gf	22/27	6/7	0/5
Extract ^b	CF#1	16/22	10/10	0/11
	X/Gf	12/14	2/2	0/3
Total		71/88	23/24	0/25

^a Bone sarcoma cultures: primary, clones, or cocultivated SC-1 or D55 cells.

^b Extracts of bone sarcomas added to Sc-1 or D55 cells.

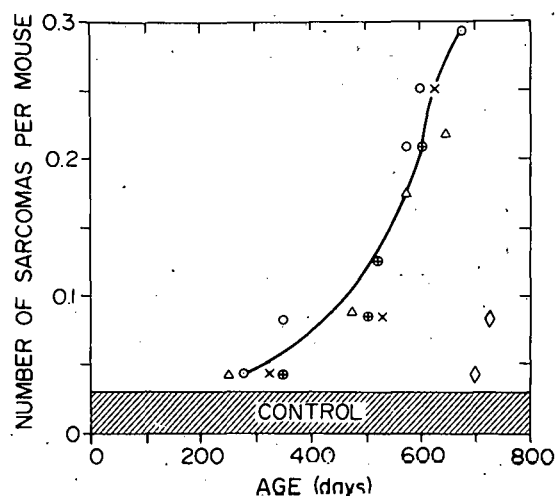


Fig. 14.3. Cumulative incidence of sarcomas in CF#1 mice as a function of time after i.p. inoculation at birth with spent tissue culture medium from ^{90}Sr -induced osteogenic sarcomas grown in cell culture. Five different groups are represented, each having received material from a different ^{90}Sr -induced osteogenic sarcoma.

ENHANCEMENT OF REVERSE TRANSCRIPTASE AND OTHER POLYMERASE ACTIVITIES BY POLYETHYLENE GLYCOL

E. W. Chan, P. J. Dale, I. L. Greco, J. G. Rose,¹ and T. E. O'Connor

Polyethylene glycol (PEG), a high molecular weight, water-soluble polymer, forms a colorless, neutral, and viscous solution in water. Among its many uses, PEG is used to concentrate bacteriophages and DNA and RNA viruses. In using the PEG concentration procedure for retroviruses, we wished to determine whether PEG had an effect on viral reverse transcriptase—an enzyme whose activity is important for virus replication and detection, and for synthesis of labeled complementary DNA probes. We found that PEG enhanced the activity of reverse transcriptases, augmenting both the rate and duration of polymerization. The effective mean molecular weight of PEG was 6000 and the optimal concentration was 12% (w/w). PEG was effective on the reverse transcriptases of all 10 Type B, C, and D viruses tested under a variety of exogenous, endogenous, and reconstitution assay systems, including the highly efficient conditions involving calf thymus DNA oligonucleotide primers. By three methods of synthesis, PEG stimulated the yield of ^3H -cDNA by a factor of 1.8-6.5. PEG did not alter the divalent cation requirements or the specificity of the enzyme. The ^3H -cDNA's made in the presence of PEG were indistinguishable in terms of size and sequence complementarity from those made

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without PEG. The stimulatory effect was partly due to the ability of PEG to stabilize reverse transcriptase and to prevent early termination. Preliminary tests indicated PEG could also stimulate other nucleotide polymerases such as the DNA-dependent DNA and RNA polymerases of *Escherichia coli* and the terminal transferase of calf thymus. The improved synthesis of specific nucleic acids attainable in the presence of PEG has significant potential applications.

ANTIVIRAL PROPERTIES OF POLY (1-METHYL-6-THIOINOSINIC ACID)

E. W. Chan, C. K. Lee, and K. Nortridge¹

The antitumor polynucleotide analogue, poly (1-methyl-6-thioinosinic acid) [poly(m¹s⁶I)], synthesized by A. Broom, Department of Medicinal Chemistry, University of Utah] is a potent inhibitor of viral reverse transcriptase. At a concentration of 10 µg/ml, the analogue totally inhibits the enzyme activity in a standard exogenous template assay involving disrupted avian myeloblastosis virions and oligo (dG)₈₋₁₂ = Poly(rC). Results of experiments varying the order of addition, the ratio of analogue to template, and determining the time course are consistent with a strong, complete, and noncompetitive type of inhibition.

Inhibition was not limited to the avian myeloblastosis viral enzyme. Poly(m¹s⁶I) also completely inhibited the reverse transcriptase of a mammalian woolly monkey simian sarcoma virus. Furthermore, it is very effective in the endogenous reaction where the natural genomic viral RNA is being transcribed in the absence of an exogenous synthetic template.

At a subtoxic level of 50 µg/ml, poly(m¹s⁶I) effectively and totally stops the production of the FBJ osteosarcoma virus by a chronically infected, high virus-producer cell line for a 2-week test period. Removal of poly(m¹s⁶I) restored virus production to its previous high titer.

In the continued presence of 50 µg/ml of poly(m¹s⁶I), susceptible D55 cells, derived from a normal Swiss mouse embryo, become resistant to infection by FBR murine osteosarcoma virus. There was potent inhibition of acute infection as determined by XC-plaque and reverse transcriptase assays for virus progeny in infected cultures.

The antireverse transcriptase and the antiviral properties of this substituted polyribonucleotide suggest that poly(m¹s⁶I) may be effective in the inhibition of the induction of osteosarcoma by FBR virus in vivo.

¹Summer 1978 participant in the Undergraduate Honors Research Participation Program, Northern Illinois University.

TEST SYSTEMS

Four carcinogen/cocarcinogen detection test systems are under development. These are (1) the quantitative measurement of the release of hydrolytic lysosomal enzymes from cultured rat alveolar macrophages; (2) the identification of morphological and biochemical changes in cultured rat tracheal rings; (3) the histochemical identification of preneoplastic liver foci; and (4) the biochemical or immunological detection of changes in chromatin that accompany neoplasia.

ALVEOLAR MACROPHAGES: ROLE IN LUNG DAMAGE INDUCED BY PARTICULATE AND NONPARTICULATE TOXICANTS

Y. E. Rahman, C. Peraino, A. M. Prapuolenis, and E. A. Cerny

The respiratory system is one of the major pathways through which air pollutants, including carcinogens, are introduced into the human body. Alveolar macrophages play a primary role in the defense mechanism against such agents. However, the biochemical nature of this defense mechanism is largely unknown; particularly unclear is the role of various hydrolytic enzymes, e.g., those of lysosomes, which are especially abundant in these phagocytic cells. The unimpaired activity of an enzymatic defense system should be essential for effective protection against lung damage induced by various airborne toxicants. Rat alveolar macrophages are obtained by lung lavage and incubated in 1640 culture medium with 5% fetal calf serum in the presence of various levels of test toxicants. In the first experiments with this technique, cell viability, as measured by dye exclusion, was reduced to 50% when alveolar macrophages were exposed for 24 hours to a 0.1 mg/ml concentration of fly ash from the pressurized fluidized bed combustor in the Chemical Engineering Division, and to 10% at a concentration of 0.5 mg/ml.

Subsequently we determined the cytotoxicity of extracts of the condensed effluent from the fluidized bed combustor (see report by Kubitschek et al. in Section 7). In this case, cells were examined for viability at different intervals not only by dye exclusion but also by their lactic dehydrogenase activity. In addition, lysosomal activation was measured by the release of acid phosphatase and β -glucuronidase into the culture medium. From the results obtained after a 24-hour exposure to condensate extracts of 10, 20, 40, and 80 μ g/ml of medium, it was concluded that lysosomes of alveolar macrophages were not specifically activated by the extracts; the decrease of acid phosphatase and β -glucuronidase in exposed macrophages was very closely correlated with the level of cell death. Further, using this alveolar macrophage system to compare two fractions of the extract that had shown different direct mutagenicities in the Ames Salmonella assay with strain TA98 (25 and 8.9 revertants/ μ g; unpublished data of H. E. Kubitschek et al.), we found that the two fractions had essentially the same effect on cell viability (LD_{50} in 24 hours of about 60 μ g/ml) and on enzyme activity.

RAT TRACHEAL RING CULTURES USED FOR THE EVALUATION OF ONCOGENIC ACTIVITY OF POLYCYCLIC AROMATIC HYDROCARBONS

N. N. Beales and C. A. Reilly, Jr.

Rat tracheal rings exposed in vitro to the chemical carcinogens benzo(a)pyrene, benzanthrane, and dimethylbenz(a)anthracene develop hyperplasia and keratinizing squamous metaplasia of the epithelium within 14 days of exposure (Table 14.2). The direct relationship between the observed histologic changes and dose of carcinogen is best illustrated by the benzanthrane data. In contrast, a noncarcinogenic analogue of benzo(a)pyrene, benzo(e)pyrene, does not induce these changes. Sham-treated controls also fail to exhibit a proliferative response. We are assessing the potential usefulness of this system for evaluating the toxic and oncogenic properties of pollutants from energy-related technologies. Results of preliminary studies using condensate of fluidized bed combustor effluent (Table 14.2) indicate that these complex mixtures of polycyclic aromatic hydrocarbons are also able to induce similar proliferation of the tracheal epithelium.

USE OF LIVER FOR THE RAPID SCREENING OF ENVIRONMENTAL POLLUTANTS FOR TUMOR INITIATING OR PROMOTING ACTIVITY

C. Peraino, E. F. Staffeldt, V. A. Ludeman, A. R. Sallese,
K. A. Rettman, and R. N. Feinstein

Tumorigenesis studies in our laboratory over the last several years suggest that liver may be sensitive to a broader range of carcinogens and tumor promoters than any other single tissue, and can therefore be used to test environmental contaminants for tumor initiating or promoting activity. Evidence is also accumulating that indicates that foci of altered hepatocytes appear in the livers of carcinogen-treated rats long before the emergence of hepatic tumors. These foci are characterized by one or more of several changes including the reappearance of enzymes normally present in fetal or embryonic liver (e.g., γ -glutamyltranspeptidase, diaphorase, alkaline phosphatase) as well as by the disappearance of enzymes normally found in mature hepatocytes (e.g., glucose-6-phosphatase and adenosine triphosphatase). It remains to be determined which of the various types of these histochemical foci are accurate predictors of subsequent tumor formation, and can thereby serve as end points in a rapid assay of environmental contaminants for tumorigenic potential. In preliminary studies, still in progress, partially hepatectomized rats were given either the known liver carcinogen diethylnitrosamine (DEN) or the polycyclic hydrocarbon carcinogen dimethylbenzanthrane (DMBA) by stomach tube. Two dose levels of each agent were used. Half the rats in each group were then placed on a diet containing 0.05% phenobarbital while the remainder were fed a control diet. Thus far, the number of foci positive for γ -glutamyltranspeptidase in the livers of these animals have been monitored, and data indicate that the numbers of such foci are directly related to DEN or DMBA dosage and that phenobarbital increases the incidence of these foci severalfold in both the DEN and DMBA groups. If these foci can

Table 14.2. Effect of Polycyclic Aromatic Hydrocarbons on Tracheal Rings
in Vitro

Test Material	Concentration µg/ml	% with Keratinizing Squamous Epithelium ^a (Number positive/Number exposed)	
		Experiment 1	Experiment 2
Benzo(a)pyrene	40	0 (0/6)	4.8 (1/21)
	20	50.0 (3/6)	14.3 (3/6)
	10	33.3 (2/6)	42.8 (10/21)
	8	0 (0/6)	--
	5	16.7 (1/6)	--
	2	0 (0/6)	9.5 (2/21)
7,12-Dimethylbenz(a)anthracene	2	0 (0/24)	--
	1	0 (0/22)	--
	0.5	4.2 (1/24)	--
Benzanthracene	40	5.6 (1/18)	--
	20	9.5 (2/21)	--
	10	30.0 (6/20)	--
	5	14.3 (3/21)	--
	2	10.0 (2/20)	--
	1	5.3 (1/19)	--
	0.5	9.5 (2/21)	--
Condensate of Fluidized	1.0	--	22.2 (2/9)
Bed Combustor Effluent	0.5	--	0 (0/15)
	0.1	16.7 (1/6)	0 (0/8)
	0.05	0 (0/6)	0 (0/9)

^a Control cultures were used in each experiment in numbers similar to those in test groups. Control rings were sham-treated with acetone in amounts ranging from 0.5 to 2.0% (v/v). In benzo(a)pyrene experiments the noncarcinogenic analogue, benzo(e)pyrene was used over the same dose range as an additional control. No proliferative response was observed in any of the controls.

be considered as truly preneoplastic, the results suggest that the liver can be used to test hydrocarbons for tumorigenic potential; previously the liver was considered refractory to the tumorigenic action of these agents. In addition, the occurrence of a dose response suggests that the monitoring of foci numbers may be useful in assessing the relative tumorigenic potency of different agents.

BIOCHEMICAL AND IMMUNOLOGICAL MARKERS OF DIFFERENTIATION AND NEOPLASIA

T. E. O'Connor, B. A. Sedita, K. A. Rettman, and K. E. Willard¹

A well-documented finding from previous research in cancer is that each tumor induced by a chemical or radiation carcinogen shows a unique profile of transplantation and cell-surface antigens irrespective of the specific inducing carcinogenic agent. In contrast, virus-induced tumors show virus-related nuclear antigens and also frequently virus structural antigens in both cytoplasm and cellular membranes. This discrepancy has posed a serious block to utilization of immunological methods in detection of carcinogen-induced cancers.

Recent literature reports suggest that either differentiation or neoplastic alteration of tissues involves changes in cellular chromatin which can be detected biochemically or immunologically, but current interpretations of these findings are limited by a number of uncertainties. We are studying the biochemical and immunological properties of chromatin from rat Novikoff hepatoma, a tumor originally induced by 4-dimethylaminoazobenzene, and normal rat liver with a view to resolving these uncertainties.

Novikoff hepatoma cells are processed under mild biochemical procedures to yield a chromatin fraction and then dehistonized chromatin and nonhistone chromosomal protein fractions are derived. Sera are raised in rabbits to intact chromatin, dehistonized chromatin, and nonhistone chromosomal proteins of Novikoff hepatoma and normal rat liver. Such sera show increased binding to the homologous chromatin or dehistonized chromatin antigens as compared to the heterologous antigens, as judged by the Levine microcomplement fixation assay. Selected sera can, in some instances, be adsorbed to the heterologous chromatin. Complement-fixation titrations of such adsorbed anti-Novikoff sera show negligible reaction with chromatin antigen of normal rat liver and a reaction pattern with Novikoff hepatoma chromatin that shows a sharp maximum and a rapid decline in regions of either excess antigen or antiserum (Figure 14.4). This complement fixation pattern resembles that obtained with other pure protein antigens and their specific antisera. In contrast, the titration pattern of the anti-Novikoff serum with Novikoff dehistonized chromatin shows a higher level of complement fixation but fails to reveal a region of antigen excess. These differences may reflect a greater

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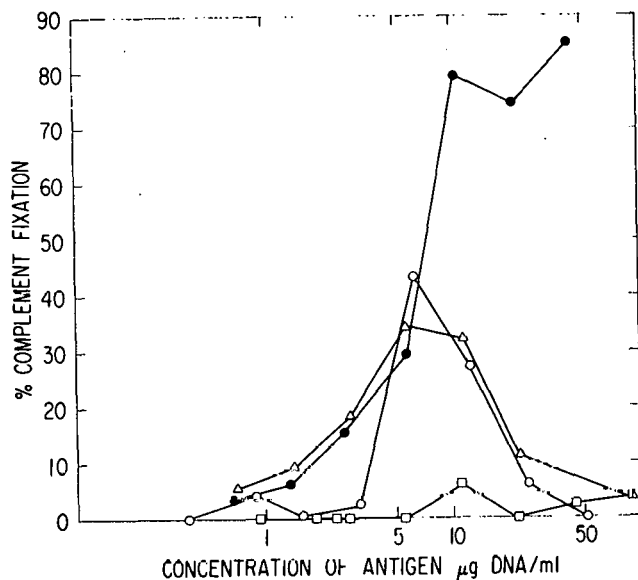


Fig. 14.4. Complement fixation by chromatin antigens with rabbit serum raised to dehistonized Novikoff hepatoma chromatin after adsorption with normal rat liver chromatin. Antigens: solid circles, dehistonized Novikoff hepatoma chromatin; open triangles, Novikoff hepatoma chromatin; open circles, chromatins from acetylaminofluorene-induced trabecular hepatocarcinomas of rat; squares, normal rat liver chromatin.

availability of the protein antigenic determinants in the dehistonized chromatin but with a reduced level of correct stereoconfiguration. Of particular interest is the observation that chromatin antigens prepared from a composite of primary rat liver tumors supplied by Dr. Carl Peraino showed a complement-fixation pattern with the adsorbed anti-Novikoff serum that coincided with that of the Novikoff chromatin antigen. These tumors resulted from exposing rats to a diet of acetylaminofluorene and were classified histologically as well-differentiated trabecular carcinomas. These findings indicate that hepatocarcinomas differing in origin and pathological classification contain an average equivalent amount of a neochromatin antigen not present in normal rat liver cells. Immunochemical and immunocytochemical procedures are now being further developed to define the molecular nature of the neochromatin antigen(s) of both normal liver and hepatocarcinomas and to explore their role in normal differentiation and carcinogenesis. The utility of antisera to these neoantigens in early detection of neoplasia arising from exposure to radiation or chemical carcinogens is also under study.

15. LIPOSOMES AS BIOLOGICAL CARRIERS

INTRODUCTION

Yueh-Erh Rahman

This program is concerned with studies seeking to develop liposomes as biological carriers for the targeted delivery of drugs. Liposomes are microscopic lipid vesicles that are formed by mixing aqueous solutions of various electrolytes or drugs with lipid materials under appropriate conditions. Larger size, multilamellar liposomes (0.2 to $> 5 \mu\text{m}$) can be prepared by shaking and smaller size, unilamellar liposomes ($< 0.1 \mu\text{m}$) are prepared by sonication.

Drugs can be incorporated into liposomes either within the aqueous center space and between the lipid bilayers of the liposomes or within the lipid bilayers. Various approaches have been used to direct liposomes to target organs, including varying the size and surface charge of liposomes, attachment of a specific antibody, or varying the lipid constituents of the liposomes.

In addition to being an attractive and versatile tool for approaching many basic problems in the field of biological research, liposomes have the potential for being useful in future clinical applications for drug delivery. At the present time, our research is centered mainly around the use of liposomes for the delivery of two categories of drugs--metal chelating agents and anti-tumor drugs.

Since October 1977, the program related to the use of antitumor drugs has been supported by a grant awarded by the National Cancer Institute. Another grant, specifically supporting the work on liposome application to iron overload, has been awarded by the National Institute of Arthritis, Metabolism, and Digestive Diseases. Although the Division's studies of liposomes are currently supported by the grant system, they are also related to the objectives of the Department of Energy. Causative substances for both metal poisoning and cancer are emitted into the environment in part as pollutants produced by various energy-related technologies.

In the last few years, since the introduction of liposomes as drug carriers, there has been a surge of interest in the development of new techniques for drug targeting. A variety of biological carriers has been introduced with the hope of achieving drug delivery to specific sites of the animal body. In our laboratory, the use of liposomes varying in lipid constituents, particularly the use of lipids that contain one or more sugar moieties, is emphasized to achieve in vivo cell targeting. Our results have shown considerable promise and we therefore plan to expand our targeting studies with glycolipid

liposomes and to identify further the different cell types in a given organ that have a specifically high affinity to a particular type of liposome. Gradient centrifugation techniques will be used to fractionate various cell types of an organ, and their uptake of a specific glycolipid liposome will be determined. Our choice of glycolipids will be largely based upon the identification by several laboratories of specific sugar moieties responsible for mediating recognition sites found on the surface of various cell systems.

Our current program therefore has two major objectives: (1) to develop liposomes with specific sugar groups that would enhance their in vivo cell selectivity, and (2) to deliver drugs by a particular type of liposome to a specific target cell.

Significant results obtained during the past year include the following. (1) Liposomes with high selectivity to spleen and bone marrow have been developed. (2) Liposome encapsulation of Ara-c resulted in a 40% survival in mice bearing a solid tumor of the lung; no controls or mice receiving nonencapsulated Ara-c survived. This is the first time that a liposome-encapsulated antitumor drug has been shown effective against a solid tumor. (3) Liposomes containing galactocerebroside were found to be significantly more effective in removing storage iron from the hepatocyte compartment of the liver, whereas liposomes without the glycolipid were more effective in removing iron from cells of the reticuloendothelial system.

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TEMPORARY STAFF DURING 1978

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LIPOSOMES IN CANCER CHEMOTHERAPY

Y. E. Rahman, K. R. Patel, M. M. Jonah,¹ and E. A. Cerny

The main objective in our use of liposomes to deliver antitumor drugs is to prepare liposomes with high selectivity for a particular organ or a particular cell type, primarily by varying the lipid constituents. So far, liposomes have been prepared with various glycolipids, and lipids extracted from various mouse organs. We have previously found that liposomes containing galactocerebroside have high uptake in the liver. We now report that the presence of esterified steryl glucoside, phosphatidyl inositol, and monogalactosyl diglyceride has increased the spleen uptake of liposome-encapsulated methotrexate by 2-, 3-, and 5-fold, respectively, compared to liposomes prepared without these glycolipids. The results also indicate that the presence of mouse kidney lipids results in a 2-fold increase of liposome uptake in the bone marrow compared to liposomes prepared without kidney lipids.

The chemotherapeutic effect of liposome-encapsulated and nonencapsulated cytosine arabinoside (Ara-c) in BDF₁ mice on Lewis lung carcinoma has also been investigated. Treatment with liposome-encapsulated Ara-c at a dose of 25 mg/kg given on Days 1, 4, and 7 after intravenous inoculation of 10⁵ live Lewis lung carcinoma cells resulted in 40 to 80% increase of the average survival over that of both the untreated controls and controls receiving Ara-c in nonencapsulated form. Intraperitoneal administration of liposome-encapsulated Ara-c was significantly more effective than either the intravenous or subcutaneous route. The average survival time for both the control groups was 25 days and there were no long-term survivals. The average survival time was increased 44% by subcutaneous Ara-c liposomes, and 54% by intravenous Ara-c liposomes, but there were no long-term survivors. In contrast, intraperitoneal administration resulted in 78% increase in average survival time, and 40% of the animals survived for at least 90 days, the cutoff point for this experiment.

LIPOSOMES IN METAL CHELATION THERAPY

Y. E. Rahman, E. H. Lau,² B. J. Wright, and E. A. Cerny

Accumulation of heavy metals in the human body, whether through the inhalation of dust particles or through the ingestion of contaminated food, has become an increasing health problem, often of a chronic nature. Our research program is concerned with the search for new therapeutic approaches that would improve the efficiency of existing chelators in removing toxic metals from the animal body. Liposomes have been used to enhance the uptake of chelators by target cells, thereby increasing the excretion of the toxic metals.

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Recently, experimental iron overload has been used as a model system to test the applicability of liposomes for removal of excess storage iron. Desferrioxamine (DF), an iron chelator extracted from Streptomyces pilosus, is used. Non-hypertransfused and hypertransfused mice are experimentally labeled with either ^{59}Fe -ferritin or ^{59}Fe -heat damaged erythrocytes (^{59}Fe -RBC's). Ferritin is known to be predominantly taken up by parenchymal cells, while damaged RBC's are mainly phagocytosed by Kupffer cells. Multilamellar and unilamellar liposomes containing DF and made of dipalmitoyl phosphatidyl choline (Dipalm-DF) and galactocerebroside (GC-DF) were prepared. Multilamellar liposomes had an average diameter of $0.5\ \mu\text{m}$ when sized in a Coulter counter. Unilamellar liposomes examined by electron microscopy after negative staining revealed an average diameter of $0.08\ \mu\text{m}$. Increased uptake and retention of DF in mouse liver was observed when DF was encapsulated in all types of liposomes, injected intravenously. The highest uptake occurred with multilamellar GC-DF liposomes, at 69% of the injected DF in liver. With multilamellar Dipalm-DF liposomes the liver uptake was 37% and with unilamellar Dipalm-DF liposomes it was 30%, while only 1.5% of the nonencapsulated DF was found in the liver.

A single intravenous injection of DF at a dose of 50 mg/kg, in Dipalm-DF or GC-DF liposomes, enhanced urinary excretion of ^{59}Fe by 3- to 15-fold from ^{59}Fe -ferritin injected mice, and 3- to 8-fold from mice given ^{59}Fe -RBC. In the ^{59}Fe -RBC model, Dipalm-DF multilamellar liposomes significantly reduced the iron content of liver, spleen, small intestine, and bone marrow. Multilamellar and unilamellar GC-DF liposomes removed more ^{59}Fe from mice injected with ^{59}Fe -ferritin than the Dipalm-DF liposomes; whereas multilamellar Dipalm-DF liposomes were more effective than GC-DF liposomes in removing ^{59}Fe from mice receiving ^{59}Fe -RBC. These observations suggest that GC-DF liposomes may have higher affinity for parenchymal cells of the liver, whereas the Dipalm-DF liposomes may have higher affinity for Kupffer cells.

Electron microscopic examination of liver sections from mice receiving either ferritin or RBC showed a surprising increase of collagen fibers. The fibers were in an unusual location, between parenchymal cells, and had a rather disordered arrangement.

16. ENVIRONMENTAL PHYSIOLOGYINTRODUCTION

George A. Sacher

The assessment of long-term effects of environmental factors has tended to focus on terminal effects such as excess cancer incidence or life shortening. The present program is focused instead on the long-term effects of energy by-products on productivity and on the integrity of the daily cycle of activity and metabolism. This is appropriate because it can be reasonably conjectured that environmental factors other than ionizing radiation are likely to have their major impacts in terms of chronic degradation of work performance and other aspects of life quality, instead of in terms of increased terminal cancer incidence. Although this program is still in an early stage, the findings to date support the hypothesis stated above.

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Russell, John J. (Scientific Associate)
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ENVIRONMENTAL INFLUENCES ON LIFE-SPAN, LIFETIME PERFORMANCE, AND DISEASE MORBIDITY

G. A. Sacher, P. H. Duffy, J. J. Russell, W. Obermeyer,¹ and R. Killeen²

Our approach to the evaluation of the effects of environmental pollutants on lifetime performance is based on four instrumentalities that are used in various combinations.

1) Genetic and constitutional differences between genotypes. To examine the genetic differences in sensitivity among individuals and genotypes within species, we have developed a diallel model in which all possible crosses of five inbred mouse strains are made and the male and female progeny of these crosses are used as experimental populations. This model system has been thoroughly defined by means of anatomical, physiological, and pathological measurements on samples of all these genotypes in a variety of environmental conditions, including the conventional laboratory environment, chronic gamma radiation fields, and a cold environment to apply a mild physiological stress to the animals. Some significant findings with this model system are presented separately below.

2) Constitutional differences between species as the basis for species differences in resistance to toxic agents. This model system employs two small rodent species, Mus musculus, the house mouse, and Peromyscus leucopus, the white-footed mouse. In our defining phase we showed that these two species differ by a factor of 2.5 in average life-span, i.e., 600 days for Mus and approximately 1450 days for Peromyscus. The use of this model is based on the hypothesis that Peromyscus leucopus is an intrinsically long-lived species, and that this long life characteristic is due to genetic factors governing the capacities for protection, regulation, and repair. This model is being used to determine whether a long-lived species differs in its response to environmental stress from a similar short-lived species. Several experiments along this line have been completed or are under way.

3) Controlled environment. An environmental chamber has been constructed that makes it possible to regulate temperature down to 0°C and control humidity within the comfort range. This enables us to put a metabolic load on the mice, and thereby to assess responses to toxic agents in a more realistic fashion than can be done in the conventional laboratory environment with its minimal metabolic load.

4) Measures of physiological performance. We have developed instrumentation that enables us to measure four variables--oxygen consumption, carbon dioxide production, motor activity, and deep body temperature--in quasicontinuous fashion for a week or longer on 12 mice simultaneously, and to log the data automatically on magnetic tape for subsequent computer analysis. This

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²Summer 1978 participant in the Undergraduate Honors Research Participation Program, Northern Illinois University.

MAT (metabolism, activity, temperature) instrument will be used to monitor physiological performance and work productivity in animals subjected to various environments. (See also the report by G. A. Sacher et al. in Section 10.)

DIEL PATTERNS OF MOTOR ACTIVITY, ENERGY METABOLISM, AND BODY TEMPERATURE

G. A. Sacher, P. H. Duffy, and J. J. Russell

A major experiment has been completed in which the 25 genotypes in the diallel genetic design (Item 2 in the preceding report) were examined in the MAT system (Item 4 in the preceding report). The 24-hour cycles of oxygen consumption were determined for male mice of each genotype and at two ages, approximately 6 months and approximately 24 months. From these data we estimated the minimum, or resting metabolic rate, the maximum rate, and the average rate over the 24-hour period, based on data from a 48-hour run for each mouse. Body weights were measured at the time of the metabolism measurements. Mean life-spans had been determined on separate samples of 20-50 of each genotype, and the subsequent survival of each mouse in the metabolism study was determined. A previous report (Sacher, G. A., and P. H. Duffy, ANL-78-90, 1978, p. 153) gives our findings on the relation of genotype mean life-span to the genotype mean levels of minimum and average metabolic rate and body weight for the 25 genotypes in the diallel design. Findings were essentially as follows. (1) There is a significant negative correlation between genotype mean life expectation and the average value of the minimum or the average metabolic rate for the genotypes. (2) There is a significant positive association of genotype mean life-span with the average body weights of the genotypes either at the young or old age of measurement. (3) A derived variable was constructed, given by the ratio of the average metabolic rate to the resting metabolic rate for each individual mouse. This variable, the activity index (AI), estimates the fraction of the total metabolic energy expended by the mouse that is utilized for motor activity and behavior in general. We found that (1) there is a significant variation between genotypes in the value of the AI, and (2) that there is a positive association between the average AI value for a genotype and its average life expectation.

In the past year we were able to assemble the data on the subsequent survival of the 85 mice used in the metabolism study. The relation of life-span to the metabolic variables was reexamined using these data, and the correlations obtained confirmed the previous study based on genotype means.

As a result of this second analysis we are now able to say that the parameters of the daily cycles of metabolism and activity of individual mice have significant capacity to predict the subsequent survival of those mice on an individual basis. This provides the basis for the design of new experimental assays of the effects of environmental pollutants on physiological rhythms and disease susceptibility.

THE EFFECTS OF A COLD ENVIRONMENT ON THE LIFE-SPAN AND TUMOR INCIDENCE OF LABORATORY MICE

J. J. Russell and G. A. Sacher

The extent to which normal environmental stressors, such as low ambient temperature, interact with environmental pollutants to influence the life-span and tumorigenesis of animals or man is unclear. The possibility exists that a cold-induced increased metabolic rate, when combined with toxic environmental pollutants, may act as a promoter of tumorigenesis. Accordingly, groups of mice from 25 genotypes obtained from F_1 crosses of five inbred mouse strains plus groups of wild Mus musculus and Peromyscus leucopus were housed in a cold chamber at 12°C for life, starting at 3 months of age. About 90% of the laboratory mouse genotypes and wild Mus are dead, whereas about 70% of the Peromyscus are still alive. The data obtained at necropsy are being analyzed to provide the following information:

- 1) A complete life table (for all mice).
- 2) The number of tumors per genotype for both males and females.
- 3) The types of tumors per genotype for both males and females.
- 4) The frequency of multiple tumors per mouse per genotype.

When completed, these data will help to elucidate whether or not the 2-fold increase of metabolic rate induced by the cold environment was expressed physiologically as life shortening and/or increased tumor incidence, and also indicate the extent of genetic variation in response to cold.

These studies will serve as a model for future experiments in which the combination of a cold-induced increased metabolic rate and a toxic environmental pollutant is investigated.

17. MOLECULAR ANATOMY PROGRAM

INTRODUCTION

Norman G. Anderson

The objective of the Molecular Anatomy Program is to provide the basic and applied research necessary to detect and measure at the molecular level the effects of radiation and of energy related mutagens, carcinogens, teratogens, and toxic agents. The scope of the work thus spans from the very basic to the applied. It includes efforts to foster development of systems and methods applicable to field measurements in nuclear or other accidents, and techniques applicable to populations at risk from low level exposure to hazardous agents.

The central functions of the human genetic apparatus (DNA) are to code for the estimated 30,000 to 50,000 protein gene products (PGP's) found in human cells and to program the expression of the genes involved. If one could examine all of the PGP's in human cells one could see how they are affected by irradiation and chemical agents at three levels: the mutational level (are there sequence alterations in PGP's?), the programming or gene expression level (are genes or gene sets switched on or off in an abnormal manner as in the case of cancer?), and the cellular level (as when PGP's are redistributed inside a cell, or leak from it). The fundamental requirement is for a very high resolution analytical method that will resolve a very large fraction (ideally all) of human PGP's. Two-dimensional electrophoresis using isoelectric focusing in the first dimension and electrophoresis in the presence of sodium dodecyl sulfate in the second has provided such a method. Each of these methods has a resolution of approximately 100, and when used two dimensionally the resolution of the system approaches the product of the two separately (i.e., 10,000). This stunning fact, made apparent by the work of O'Farrell, and the development of two-dimensional electrophoresis into the ISO-DALT system at Argonne (Anderson, N. G., and N. L. Anderson, *Anal. Biochem.* 85, 331, 1978; Anderson, N. L., and N. G. Anderson, *Anal. Biochem.* 85, 341, 1978) makes possible the efforts described in the following reports.

The Molecular Anatomy Program is concerned with the following studies:

- 1) Development of analytical systems. To develop high-resolution two-dimensional electrophoresis into a clinically useful system capable of handling large numbers of samples it will be necessary to examine in detail the theoretical basis of the separations and the factors limiting resolution. Also required are methods for calibration and precise spot localization and identification, methods for reliable sample identification, techniques for extending the limits of detection, automation, and computerized image analysis and data reduction. All are now under investigation or development.

2) Mapping of human protein gene products. High-resolution PGP maps of human body fluids, tissues, and individual cell types are basic to this program. Progress is being made in mapping plasma, muscle, red cell lysates, lymphocytes, brain tissue, saliva, seminal fluid, and hair follicle cells. Methods have been developed for spot identification using the centrifugal fast analyzer in concert with methods for protein fractionation, including heat denaturation.

3) Mutation rate monitoring. To use charge shifts for mutation rate studies it is necessary to demonstrate that single charge differences can be detected in each spot examined, and methods for doing this have been developed. The number of samples required for mutation rate estimates or to detect changes is inversely proportional to the number of PGP's examined. Present high resolution methods drastically reduce the number of samples required for analysis.

4) Cancer-associated changes. Mapping of cancer cells allows PGP's characteristic of malignancy to be discovered and characterized. The objective is to find indicators either of cancer or precancerous states useful for screening. Thus far the problem is not to find potential indicators--rather the problem is to determine which ones will be useful.

5) Urinary protein mapping. Cytotoxic injury as distinguished from genetic damage almost invariably produces cell leakage, and many of the proteins leaked are small enough to pass the kidneys and appear in the urine. Tumor cells are also known to shed proteins and some of these also find their way into urine. Hence urine contains a surprising number of potential indicators of disease and of organ damage. The diagnostic possibilities inherent in these facts have not previously been fully exploited because suitable analytical methods were lacking.

Translation of the promising findings obtained thus far into use in clinical laboratories and in laboratories concerned with screening populations at risk from nuclear accidents, fallout, or exposure to injurious chemical agents will require a variety of efforts in basic chemistry, biology, optics, mechanics, electronics, photoprocessing, image analysis, and computerized data reduction. These efforts we have now defined. Over 16,000 two-dimensional gels have now been run, several collaborative laboratories in clinical settings have been organized, and considerable progress has been made in the transfer of our technology to industry.

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DEVELOPMENT OF TECHNIQUES FOR HIGH RESOLUTION PROTEIN MAPPING

N. L. Anderson and B. J. Hickman

Methods for the two-dimensional electrophoretic mapping of radiolabeled human lymphocyte proteins were improved, and initial family studies have been undertaken. An average heterozygosity for major cellular proteins was obtained that is far lower than that observed in the plasma proteins and the house-keeping enzymes considered by classical human genetics.

Methods for the isolation of immunoglobulins from serum using protein A Sepharose microcolumns were developed and applied to the two-dimensional analysis of mouse and human immunoglobulins. Groups of inbred mice raised in the same cage were shown to give highly (70-80%) reproducible light chain patterns of about 100 spots, while the light chain pattern in outbred mice (as in Figure 17.1) or in humans was shown to consist of a much larger number of unresolved components.

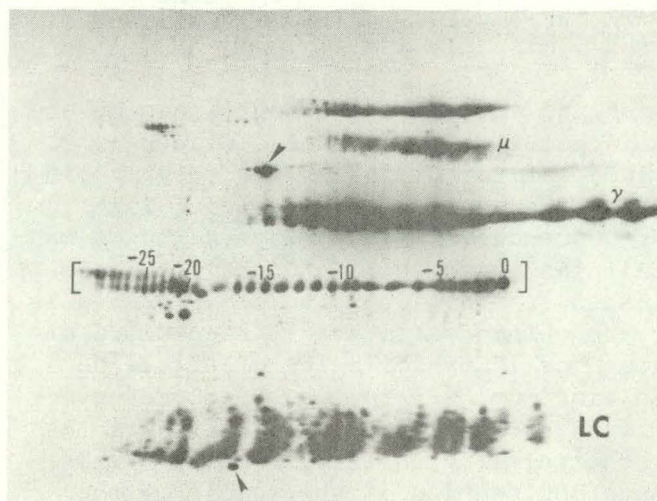


Fig. 17.1. High resolution two-dimensional (ISO-DALT) separation of serum immunoglobulins from a wild white-footed mouse, *Peromyscus* sp. LC, light chains; μ , γ are heavy chains of IgM and IgG; downward pointing arrow indicates contaminating albumin and upward pointing arrow indicates Apo A-1 lipoprotein. The spots in the numbered horizontal row are creatine phosphokinase carbamylation charge standards.

A technique for progressive thermodenaturation of protein mixtures was developed and applied to the analysis of soluble proteins from rabbit muscle and human leukocytes. By comparing enzyme activity profiles with abundances of spots on two-dimensional gels, it was possible to correlate specific activities with specific protein spots. It was further found that addition of substrate thermostabilized specific enzymes and made possible identification of classes of spots, for example spots binding NADH, without regard to specific function. In the course of this work, it was discovered that β and α cytoplasmic actins bind ADP differently. This is the first functional difference that has been found between these analogous and ubiquitous proteins.

In order to standardize the isoelectric focusing dimension of the ISO-DALT two-dimensional system, a series of charge-shift pI standards was developed. These now allow assignment of a reproducible, internally standardized isoelectric position to each protein in such a separation.

VERY HIGH SENSITIVITY PROTEIN STAINING

S. L. Nance

We have modified a method for silver staining of proteins in acrylamide gels (Switzer, R. C. III, C. R. Merrill, and S. Shifrin. A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels. *Anal. Biochem.*, in press) in several ways to decrease cost and operator exposure to toxic chemicals. The technique is approximately one hundred times more sensitive than the standard method of staining gels with Coomassie Blue. Because some tissue proteins are present in only minute amounts and others apparently do not stain with Coomassie Blue, silver staining is proving quite useful in extending the limits of detection of proteins in the ISO-DALT system.

TWO-DIMENSIONAL ELECTROPHORETIC MAPPING OF BASIC PROTEINS: THE BASO-DALT SYSTEM

K. E. Willard¹ and N. G. Anderson

High-resolution separations of proteins and protein subunits can be obtained by two-dimensional polyacrylamide gel electrophoresis. O'Farrell et al. (*Cell*, 12, 1133, 1977) have described a first dimension electrophoresis procedure to resolve basic proteins which they call a NEPHGE (nonequilibrium pH gradient electrophoresis). In this procedure, improved separation of many basic proteins resulted from loading samples at the acidic end of the focusing gel and running for 1600 volt-hours instead of the 10,000 volt-hours used in isofocusing. We were unable, however, to obtain satisfactory resolution of the basic proteins from Novikoff hepatoma cells by following O'Farrell's procedure; the histones were not solubilized by O'Farrell's sodium dodecyl sulfate (SDS) and NP-40 lysis buffers, and a considerable proportion of the total protein applied failed to enter the gel (Figure 17.2, Part B). This phenomenon could be due to inadequate solubilization of the sample by the SDS/NP-40 method and/or the inability of the sample to enter the gel because of the attached SDS. In our system, samples for the first dimensional electrophoresis (BASO) are solubilized in a mixture of 0.5% dipalmitoyl L- α -phosphatidyl choline (PC), 9.5 M urea, 2% ampholines, and 1% mercaptoethanol with the pH adjusted to 2.8 with H_3PO_4 . The samples are then centrifuged for 1 hour at 20°C and 50,000 rpm ($\omega^2 t = 85 \times 10^7$) to sediment all particles with an S value greater than 12. After electrophoresis for 3200 volt-hours (Figure 17.2, Part A), we obtained more complete patterns of the basic proteins (including the histones) in Novikoff hepatoma cells. This system is called the BASO-DALT system, since the second (SDS) dimension is identical with that used in the ISO-DALT system.

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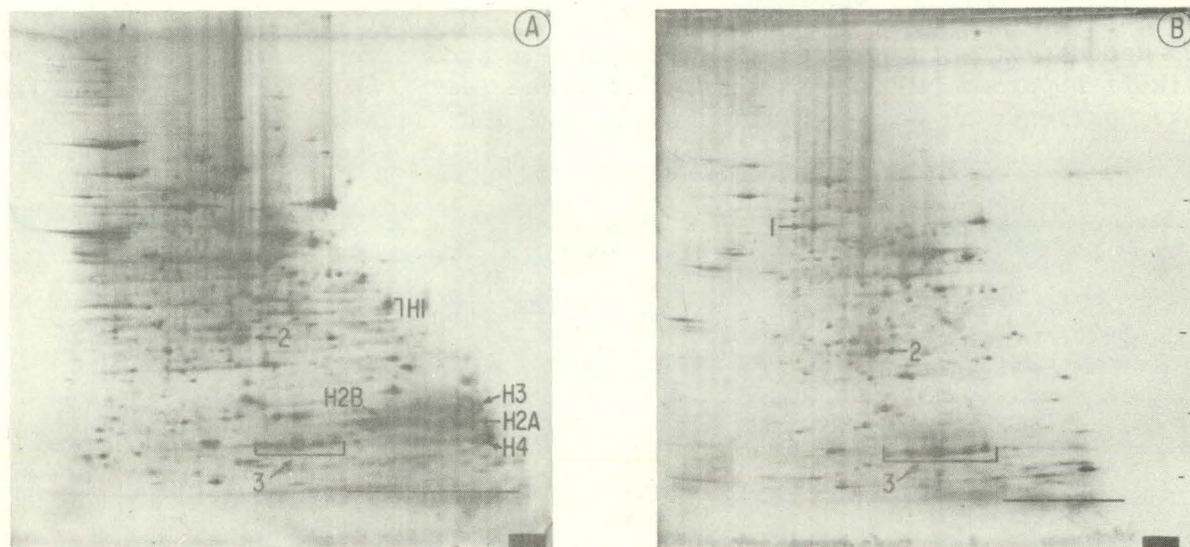


Fig. 17.2. Two dimensional electrophoresis patterns of Novikoff hepatoma cells illustrating different methods of solubilization. Samples were electrophoresed for 3200 volt-hours using LKB 3.5 - 10 ampholines in the ISO-DALT apparatus. The gels are oriented with the basic end to the right and the acidic end to the left. (A) Cells were lysed in the PC/urea mixture, allowed to stand at room temperature for 1 hour, and then centrifuged for 1 hour at 50,000 rpm. (B) Cells were mixed 1:1 with the SDS lysis buffer described by O'Farrell and allowed to stand for 10 min. This mixture was then diluted 1:1 with O'Farrell's NP-40 lysis buffer; the final protein concentration equaled that of the samples in A. The histones H1, H2A, H2B, H3, and H4 are identified in A. Selected proteins are labeled 1-3 for reference purposes in both A and B. The area above the bar represents the greatest difference between the gels.

Isoelectric point standards for the ISO-DALT system based on carbamylation of proteins by heating at 95°C in 9.5 M urea have been described (Anderson, N. L., and B. J. Hickman, *Anal. Biochem.* 93, 312, 1979). Carbamylated creatine phosphokinase (CK) has been chosen as a provisional standard for the ISO-DALT system (see Figure 17.1). However, because the isoelectric point of CK is about 6.1, the most basic species migrates only about halfway across the BASO gel. It was necessary to develop new, more basic isoelectric point standards for internal standardization of the BASO system. Carbamylation mixtures of glyceraldehyde-3-phosphate dehydrogenase yielded trains of well-defined, evenly spaced spots which run about halfway down the SDS slab gel. Although minor spots appear between the first three charge shifts, and two minor trains with higher SDS molecular weight values appear toward the acid end of the gel, this protein yields a train of spots suitable for internal standardization of the BASO system.

In addition to Novikoff hepatoma cells, the BASO-DALT system has been used to examine the basic proteins in muscle samples, concentrated urinary proteins, liver proteins, and serum proteins. Utilizing this system we have demonstrated that human urine contains proteins that retain their biological activity

and can stimulate de novo protein synthesis in Novikoff hepatoma cells. Figure 17.3 demonstrates the concentration-dependent production of these proteins in Novikoff hepatoma cells. This stimulatory activity was detected in urine from cancer patients as well as urine from normal individuals.

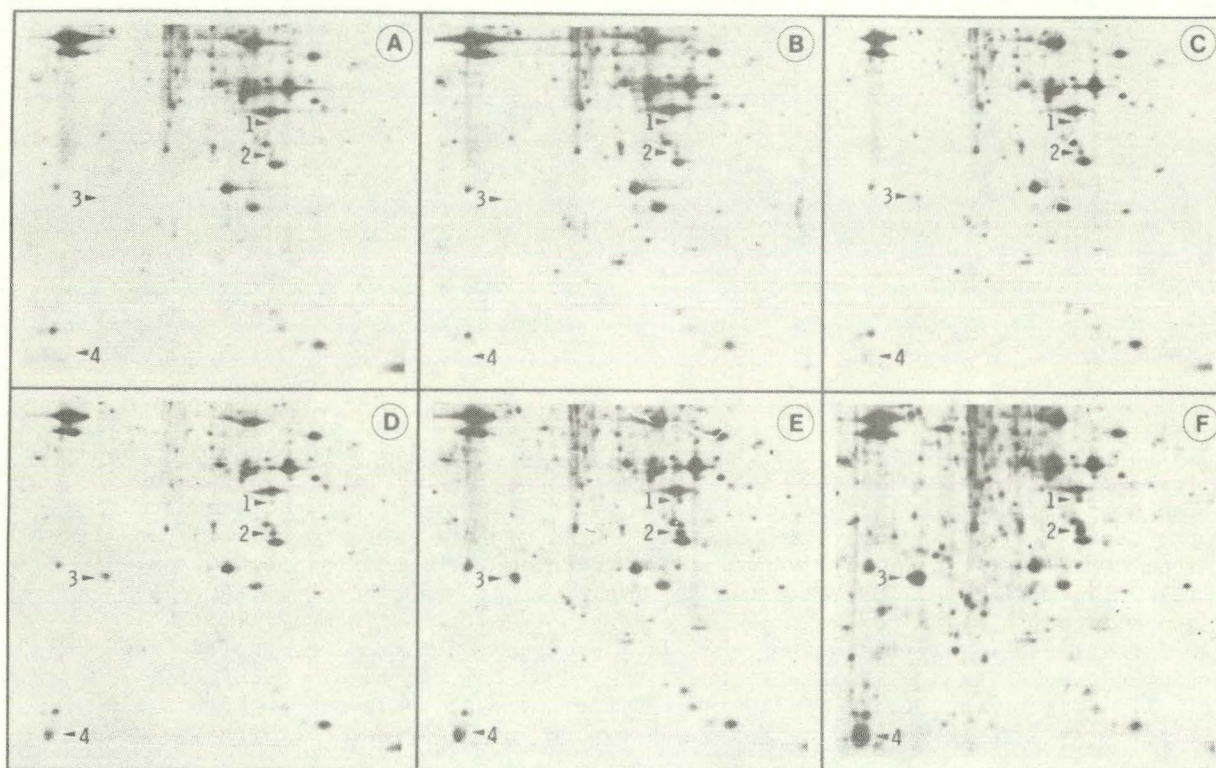


Fig. 17.3. Each panel represents a portion of a BASO-DALT pattern of Novikoff hepatoma cells cultured with increasing concentrations of human urinary proteins. Cells were cultured for 48 hours in serum-free medium 479 containing ^{35}S -methionine. Radioactive cell samples were solubilized as described in Figure 17.1A. Samples were electrophoresed for 4000 volt-hours using LKB 3.5 - 10 ampholines. The second dimension gels were dried and then placed on film for 10 days. Autoradiographs of cells cultured with increasing concentrations of urinary proteins, are as follows: (A) control, (B) 0.0001 mg/ml, (C) 0.001 mg/ml, (D) 0.01 mg/ml, (E) 0.1 mg/ml, and (F) 1.0 mg/ml. The gels are oriented as in Figure 17.1. Selected proteins are labeled 1 to 4 for cross reference.

The BASO-DALT technique should provide a better means for analysis of basic protein mixtures found in tissues and cells. Except for the histones, there have been relatively few studies of these basic proteins. Use of the conventional ISO procedure and the BASO technique described here provides a comprehensive mapping technique, showing histones as well as other basic proteins necessary to catalog human protein gene products.

TWO-DIMENSIONAL ELECTROPHORETIC ANALYSIS OF HUMAN URINARY PROTEINS

N. G. Anderson, S. L. Tollaksen, and N. L. Anderson

Human urine contains small amounts of protein which may be classified into eight groups, according to origin: (1) normal plasma proteins or fragments of them, (2) proteins originating in the kidney, (3) proteins arising in the remainder of the urogenital tract, (4) tissue leakage proteins reaching the urine through the circulation, (5) hormones or other signal substances, (6) proteins produced by the placenta or fetus, (7) tumor-associated proteins, and (8) products of bacterial or viral infection. Several hundred proteins are resolved using two-dimensional electrophoresis to analyze human urinary protein concentrates; hence the possibility exists for finding among them indicators of a wide variety of types of disease or injury. The effects of energy-related chemical pollutants and radiation would include the presence of tissue proteins in urine leaked from damaged tissues, presence of new mutant proteins not found in either parent, proteins indicating cancerous or precancerous conditions, or alterations in the ratio of high and low molecular weight proteins due to glomerular or tubular damage (the latter being often due to heavy metal intoxication). Methods for concentrating urinary proteins and for mapping them two-dimensionally have been developed and applied to a variety of urines. As expected, marked alterations are found in kidney injury, in several types of cancer, and after irradiation. This survey work indicates the importance of detailed study and identification of as many proteins as possible, and the development of scanning systems that will permit quantitative analysis.

TWO-DIMENSIONAL ELECTROPHORETIC MAPPING OF MUSCLE PROTEINS

C. Smith, N. L. Anderson, and N. G. Anderson

The objectives of this work are to develop clinically useful methods for the analysis of human muscle biopsy material by two-dimensional electrophoresis and to identify the proteins separated by this technique. Using rabbit psoas muscle as a model, we have developed methods for sample acquisition and preparation. A modification of the first dimension separation in the ISO-DALT system is sometimes used for two-dimensional electrophoresis. The separation in the first dimension is either by isoelectric focusing (ISO, for resolution of acidic and neutral proteins) or by nonequilibrium pH gradient electrophoresis (NEPHGE, for resolution of neutral and basic proteins), and is then followed by separation in the second dimension using sodium dodecyl sulfate (SDS) electrophoresis, as in the ISO-DALT system. The peptide components of ten enzymes and of myosin, actin, tropomyosin, and troponin have been identified in the rabbit psoas muscle two-dimensional electrophoresis patterns.

Figure 17.4 shows the two-dimensional electrophoresis pattern when isoelectric focusing is used for the first dimension separation. Figure 17.5 shows the same sample separated by the NEPHGE technique in the first dimension. In the NEPHGE, samples are loaded at the acidic end of the first dimension gel instead of the basic end as in the case of the ISO technique. This technique

results in higher resolution of basic proteins as can be seen by comparing the aldolase (ALD) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) patterns in Figures 17.4 and 17.5. The NEPHGE also permits detection of very basic proteins such as myokinase (MK) and the inhibitory subunit of troponin (TnI) (Figure 17.5) which do not enter gels when isoelectric focusing is used for first dimension separation (Figure 17.4). Identification of proteins in two-dimensional electrophoresis patterns of rabbit muscle is being used as the foundation for the mapping of human muscle proteins. Preliminary work has shown definite similarities in the patterns of rabbit and human skeletal muscle and some tentative identifications in the human patterns have been made (Figure 17.6).

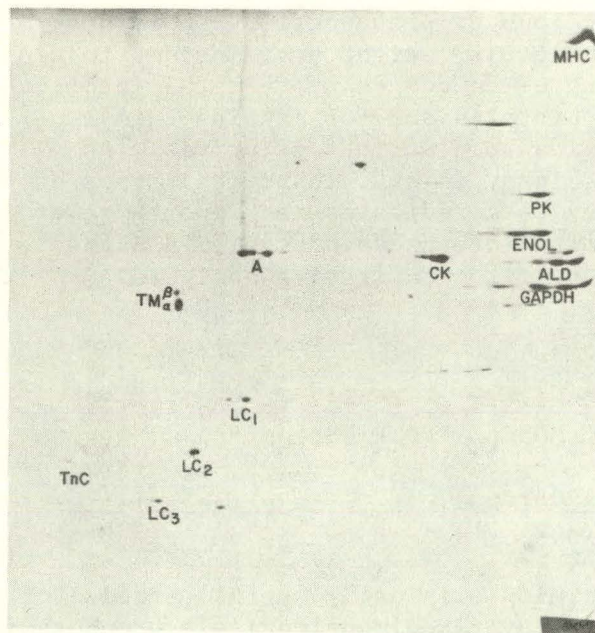


Fig. 17.4. Two-dimensional patterns of rabbit psoas muscle whole homogenate (200 μ g protein) using isoelectric focusing in the first dimension followed by SDS electrophoresis. Isoelectric focusing was done using Brinkman 2-11 ampholines in the ISO-DALT apparatus. The gel is oriented with the acidic side to the left and the basic side to the right. A, actin; α TM and β TM, tropomyosin; MHC, myosin, heavy chain; LC₁, LC₂, and LC₃, myosin light chains; TnC, calcium-binding troponin subunit; PK, pyruvate kinase; CK, creatine kinase; ENOL, enolase; ALD, aldolase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

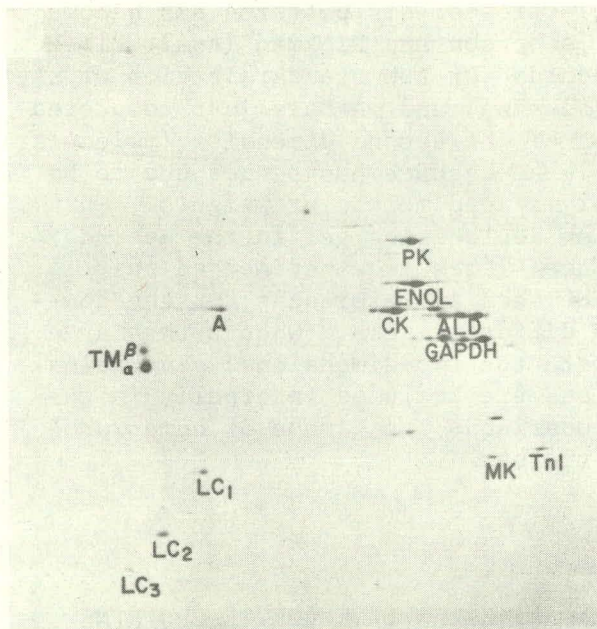
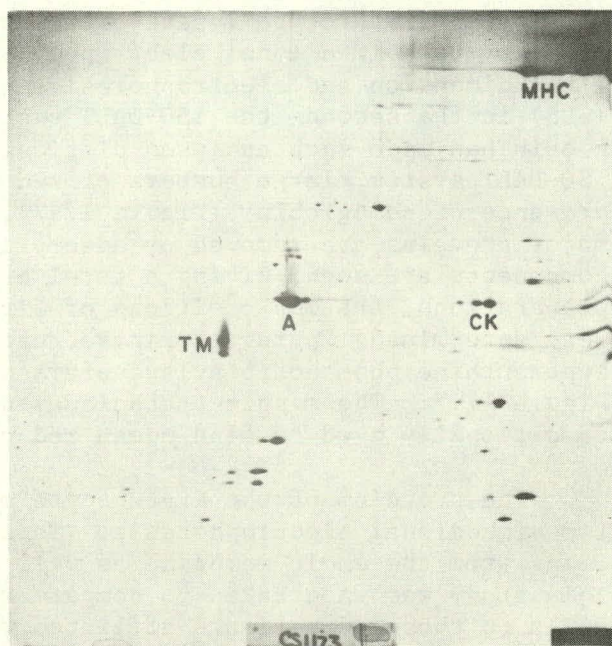


Fig. 17.5. Two-dimensional patterns of rabbit psoas muscle whole homogenate (200 μ g protein) using nonequilibrium pH gradient electrophoresis (NEPHGE) in the first dimension. Samples were separated in the first dimension using the modified NEPHGE technique in gels containing Brinkman 2-11 ampholines. After electrophoresis for 4000 volt-hours, the proteins were separated in the second dimension using sodium dodecyl sulfate electrophoresis. The gel is oriented as in Figure 17.4. The symbols are as identified in Figure 17.4, with the addition of the following: TnI, inhibitory troponin subunit and MK, myokinase.

Fig. 17.6. Two-dimensional electrophoresis pattern of human skeletal muscle whole homogenate. A human muscle biopsy sample was analyzed using isoelectric focusing in the first dimension. Two hundred μ g of protein was applied to the isofocusing gel. The symbols are as identified in Figure 17.4 (TM = tropomyosin), and the gel is oriented as in Figure 17.4.



Standardization of two-dimensional electrophoresis patterns has become important for the comparison of samples. Anderson and Hickman (Anal. Biochem. 93, 312, 1979) have described charge standards for the standardization of the first dimension separation (isoelectric focusing) and we have just completed development of markers for standardization of the second dimension (molecular weight separation). Rat heart muscle whole homogenate has been found to be a reliable source of proteins having evenly spaced molecular weights. When this mixture is used in combination with an isofocusing gel in the second dimension separation, a pattern of horizontal lines is superimposed over the final spot pattern. These lines can be used as references for the comparison of different samples separated on different gels. When both charge standards and rat heart muscle are used with the two-dimensional separation of a sample, coordinates for both dimensions are included internally in the gel. This technique will be helpful in comparisons among maps of human protein gene products from muscle and other tissues.

RED CELL PROTEINS: TWO-DIMENSIONAL MAPPING OF HUMAN ERYTHROCYTE PROTEINS

J. J. Edwards, N. G. Anderson, S. L. Nance, and N. L. Anderson

Human erythrocyte lysate proteins were resolved into over 250 discrete spots by two-dimensional electrophoresis using isoelectric focusing in the first dimension and electrophoresis in the presence of sodium dodecyl sulfate (SDS) in the second--the ISO-DALT system. The overwhelming excess of hemoglobin has made such analyses difficult in the past. However, with the ISO-DALT system, large numbers of red cell proteins can be mapped even in the presence of hemoglobin (Figure 17.7). When hemoglobin and several other major proteins are removed by adsorption to DEAE-cellulose, additional minor components are seen, giving a total of over 275. With the use of purified preparations, the map positions of five red cell enzymes or their subunits were determined: pyruvate kinase, catalase, glucose-6-phosphate dehydrogenase, hypoxanthine phosphoribosyltransferase, and carbonic anhydrase, as shown in Figure 17.7. The mapping techniques described complement and extend those traditionally used to find human red cell protein variants.

The proteins of the erythrocyte membrane are currently being analyzed by two-dimensional electrophoresis. This study involves the separation of proteins from the whole membrane as well as the analysis of purified components. This study was undertaken to compare the proteins of membranes from normal cells to those of patients afflicted with Duchenne muscular dystrophy.

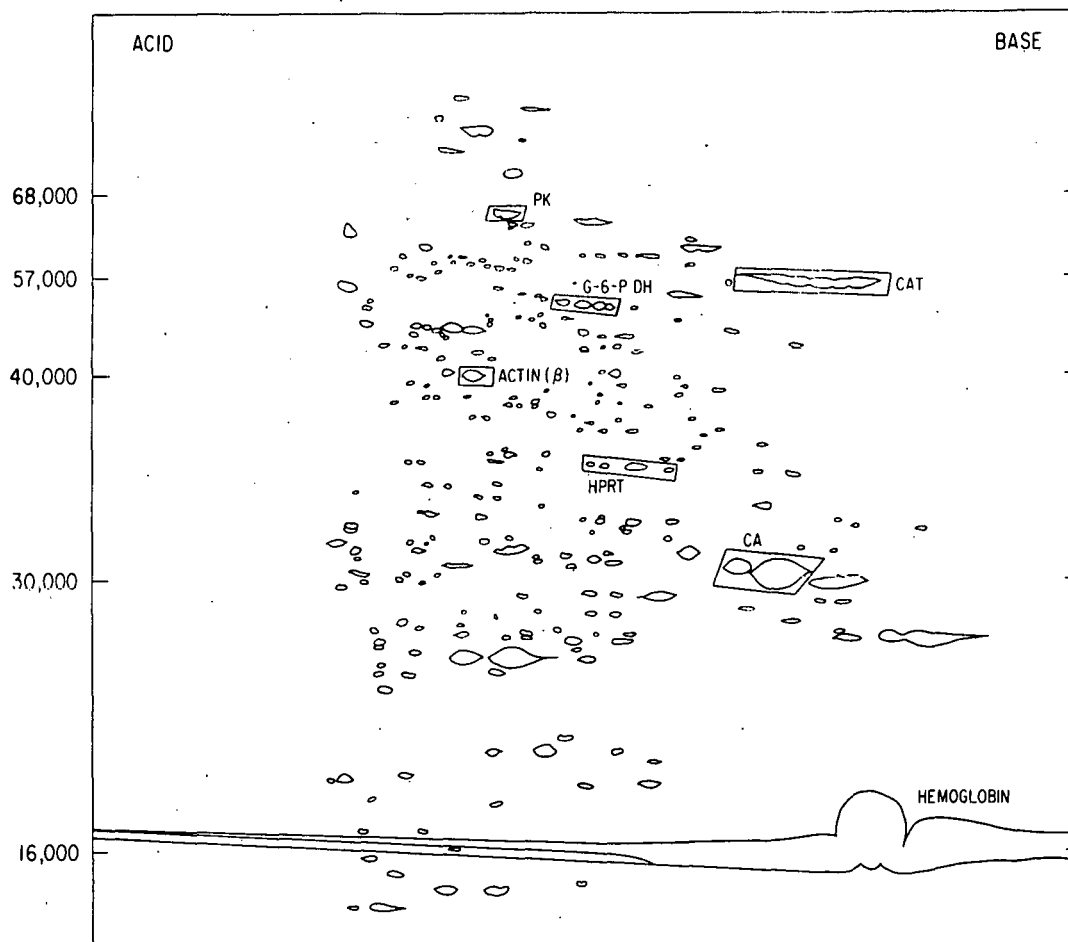


Fig. 17.7. Two-dimensional electrophoretic separation of human erythrocyte lysate proteins. The sample was 12.5 μ l of fresh lysate denatured in an equal volume of buffer containing 2% SDS/5% dithiothreitol. Isoelectric focusing was from right to left and molecular weight separation from top to bottom. The map locations of known erythrocyte enzymes are labeled. CAT = catalase; PK = pyruvate kinase; G-6-P DH = glucose-6-phosphate dehydrogenase; HPRT = hypoxanthine phosphoribosyltransferase; CA = carbonic anhydrase. Hemoglobin and actin are also labeled. Molecular weight values (ordinate) were determined by SDS electrophoresis of known proteins.

COMPUTERIZED REDUCTION OF TWO-DIMENSIONAL GEL DATA

N. L. Anderson, John Taylor, Jr.,¹ B. S. Coulter,¹ A. Scandora,²
and M. Kempf²

The human eye can accurately compare two complex patterns in great detail, but the mind cannot readily deal with comparisons among large numbers of such patterns. For this reason, it has proved essential to develop a computerized system for cataloguing and quantitating the thousands of spots visible on two-dimensional (ISO-DALT) protein gene product maps. During 1978, work was begun on such a system based on a PDP 11/60 minicomputer, with image input from a high-speed, two-dimensional microdensitometer (Optronics P-1000) and image display on a 512 x 512 element color television (Grinnell GMR-27). The system is designed to find and quantitate all the spots in a pattern (after initial smoothing and background subtraction), using a two-dimensional Gaussian fitting technique. It is anticipated that the ability to measure and intercorrelate the abundances of thousands of proteins in a variety of types of cells and body fluids will enable us to explore gene regulation, differentiation, and cancer in much greater detail than has heretofore been possible.

¹Applied Mathematics Division.

²Science Applications, Inc.

18. THE ARGONNE BIOANALYTICAL CENTER FOR THE STUDY OF STABLE ISOTOPE APPLICATIONS IN HUMAN HEALTH AND DISEASE

INTRODUCTION

Peter D. Klein

This program has five major aspects: first, the development of analytical instrumentation of adequate sensitivity, stability, and simplicity to conduct stable isotope measurements in a routine manner; second, the development of appropriately labeled compounds for metabolic investigations, initially through custom syntheses but eventually to be made available through commercial sources; third, development of analytical methodology to isolate, purify, and determine the isotopic content of specific organic compounds reflecting metabolic or disease states; fourth, collaborative development of clinical applications and testing, on a routine basis, through a network of clinical centers around the country; and finally, the collection and dissemination of stable isotope information on an international scale through survey publications and conferences.

A new, more sensitive gas chromatographic system for analysis of hydrogen, carbon dioxide, oxygen, and nitrogen in breath has been developed. This instrumentation is used in the detection and quantitation of carbohydrate malabsorption. We have shown that relating the observed level of hydrogen to the concentration of carbon dioxide in the same sample achieves a significant reduction in the variability of this measurement, and reduces the dependence of values on the breath collection technique.

New procedures for the quantitation and study of tracer kinetics of methadone and of taurine have been developed. In the first instance, three isotopic variants of the unlabeled compound are used to determine the endogenous level of the unlabeled drug and to provide independent measurements of the rates of metabolism of the individual optical isomers. In the second instance, procedures for the recovery and measurement of isotopic abundance in the putative neurotransmitter taurine have been developed. The complete failure of previous efforts in this area has been shown to be due to a novel "invisibility" of taurine derivatives in conventional gas chromatographic detection systems, which is overcome by mass spectrometry.

Studies of bile acid kinetics with ^{13}C -labeled bile acids in pregnant women, seeking to find the basis of an increased cholelithiasis (gallstone formation) in this population have shown that, contrary to expectations, the total quantity of bile acids in the enterohepatic circulation is increased during the first and second trimester of pregnancy. Similar studies are also under way to investigate the effect of oral contraceptive steroids in women of childbearing age.

Stable isotopes of metals are potentially powerful tools to be used in studying trace metals that are essential for human nutrition or that are toxic by-products from energy production. We are currently developing methods for the isolation of metals and for the determination of their isotopic abundance.

The activities of this program contribute to the medical applications of stable isotopes as nonradioactive, noninvasive probes of specific metabolic processes that may be affected by environmental contaminants. This program provides a means of studying the metabolism and fate of pollutants in the examination of disease states and lays the basis for estimating levels of human exposure through clinical studies of selected populations. These studies can be extended into epidemiological examinations of occupational and general populations as the techniques prove to have significant diagnostic values.

Finally, this program represents the confluence of a number of research programs within DOE and also in various institutes of the National Institutes of Health, and it serves to link the network of stable isotope users within a national and an international framework.

REGULAR STAFF

Hachey, David L. (Chemist)
Klein, Peter D. (Senior Biochemist)
Szczepanik-Van Leeuwen, Patricia A. (Biochemist)

TEMPORARY STAFF DURING 1978

DeMark, Bruce R. (Postdoctoral Appointee)
Irving, Charles S. (Postdoctoral Appointee)
Niu, Hsien-chi (Postdoctoral Appointee)
Stellaard, Frans (Research Associate)
Tserng, Kou-Yi (Postdoctoral Appointee)

INSTRUMENTATION

D. A. Schoeller¹ and H. C. Niu

The automated CO₂ purification system and isotope ratio analyzer has been installed in the Stable Isotope Facility at The University of Chicago Hospitals and Clinics. As of January 1979, it has been in use for the analysis of breath CO₂ for 7 months as part of the ¹³CO₂ breath test program. The instrument has been reliable, analyzing between 30 and 40 samples per day with operator intervention only at 4-hour intervals. Cost and time of the analysis are comparable with scintillation counting of ¹⁴CO₂.

Our dual source gas chromatograph-mass spectrometer (GC-MS) has been upgraded with the addition of a molecular separator which reduces the helium flow into the ion source. The reduced helium flow has improved operation of the electron impact ion source and resulted in greater sensitivity and stability of operation. The copper gas lines used for introduction of reagent gas during the chemical ionization mode have been replaced with stainless steel. The stainless steel lines enable the use of reagent gases, such as ammonia, that would corrode the copper lines.

ORGANIC SYNTHESSES

D. L. Hachey and K. Y. Tserng

Synthetic preparation work has been substantially curtailed by the departure of Dr. K. Y. Tserng to a permanent position elsewhere. Before the conclusion of his appointment, he prepared several large scale batches of 24-¹³C-labeled bile acids for the bile acid kinetic studies currently under way. In addition, work was completed on the synthesis of the 7- and 12-mono-sulfates of bile acids, and their glycine and taurine conjugates. This work, which concludes the syntheses of bile acid sulfates, has permitted the determination of their chromatographic mobilities and separation characteristics. In addition, they have been used by Dr. Alan Hofmann (University of California, San Diego) to ascertain the forms of sulfated chenodeoxycholic acid formed in human studies. A large scale batch of methacetin-¹³C was synthesized to provide sufficient material for approximately 100 clinical studies. Finally, several Schiff base chelating agents were prepared to test their suitability for the chromatographic separations of zinc and copper. Using these and other chelating agents, a number of metal chelates have been prepared for magnesium, calcium, iron, copper, zinc, and cadmium to test their suitability for mass spectral isotope ratio measurements.

¹Resident Associate, Division of Biological and Medical Research, and Research Associate (Assistant Professor), The University of Chicago.

ANALYTICAL METHODOLOGY

The following reports present the progress made during the year in the development of analytical methods designed to solve problems in using stable isotopes in specific biological and clinical applications.

STUDIES WITH STABLE ISOTOPES OF METALS

D. L. Hachey and J. C. Blais¹

Stable isotopes of metals are potentially powerful tools for studying trace metals that are essential for human nutrition or that are toxic by-products from energy production. Techniques have been developed to wet chemical ash biological samples using strong oxidizing acids, since the biological matrix in which metals are entrained interferes with mass spectrometric analysis. Wet chemical ashing is adequate for studying the more abundant elements, such as magnesium, calcium, iron, copper, and zinc which are of interest in nutritional studies. Another problem is to identify chelating agents that efficiently extract metal ions from aqueous solution and that, in addition, have good mass spectral properties, e.g., good volatility and stability, convenient ions for isotope ratio studies, and freedom from isotope memory effects.

For plasma and urinary magnesium and calcium, mass spectral studies have been conducted using a fluorinated β -diketone as the chelating agent. For the alkaline earth series, electron ionization mass spectrometry (EI-MS) is most useful; under chemical ionization (CI-MS) conditions only ions related to the free chelating agent are observed. Preliminary isotope ratio studies using the PE-270 mass spectrometer indicate a severe isotopic memory effect, which apparently is due to isotope exchange with insulating material used in manufacture of the direct insertion probe. Use of an all-glass system should eliminate the problem. Both magnesium and calcium isotope ratio studies indicate that it is possible to obtain precise isotope ratio measurements ($\pm 0.06\%$) and to obtain accurate natural abundances (± 0.2 - 0.8%) after correction for isotope contributions from the chelating agent.

For analysis of transition metals (iron, copper, zinc) a much wider selection of chelating agents and ionization techniques is available. Fluorinated β -diketones have only limited use because of extensive isotopic memory effects; the only notable exception is chromium trifluoroacetylacetonate. One must resort to various exotic chelating agents containing oxygen, nitrogen, and sulfur ligands, or use the more stable organometallic compounds. Ferrocene, an organometallic derivative of iron, has ideal properties for isotope ratio analysis by GC-MS; however, it is difficult to prepare

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on a microgram scale. Ferrocene is best analyzed by EI-MS; under CI-MS conditions it contains a mixture of ions formed by proton transfer (MH^+) and by charge exchange oxidation to the ferricinium ion (M^+). Copper can be analyzed as a fluorinated Schiff base chelate, using isobutane CI-MS. Zinc is best analyzed using chelating agents containing sulfur, such as diethyldithiocarbamate (DDC) or monothio-trifluoroacetylacetone. There is some difficulty with an isotopic memory effort when $Zn(DDC)_2$ is analyzed by CI-MS, but the problem seems to be small under EI-MS conditions. Isotope ratio studies have achieved a precision of ± 0.05 - 0.30% for simultaneous measurement of all five naturally occurring zinc isotopes. Similarly, we have obtained experimental precision of ± 0.12 - 0.72% for simultaneous measurement of all eight cadmium isotopes by CI-MS. Absolute accuracy for a cadmium natural abundance isotopic standard ranges between ± 0.01 - 0.48% , corrected for isotopic contributions from the chelating agent. Similar experimental accuracy and precision results have been obtained for iron trifluoroacetylacetonate, but this compound is unsuitable for isotope tracer studies by CI-MS because of exchange with iron used in the manufacture of ion source components.

METHADONE PHARMACOKINETICS

D. L. Hachey and K. Nakamura¹

Multiple isotopic tracer studies for plasma methadone pharmacokinetics required improved isolation techniques for recovering methadone from plasma. Dr. Kanichi Nakamura has developed techniques for isolating methadone using a short, disposable C₁₈-silica gel cartridge. The extraction procedure is more rapid, drug recoveries have been improved nearly 2-fold, and purity has been increased by 5-fold. Techniques have been refined to the point where plasma drug level, multi-isotope kinetic studies are routinely possible. Novel chemical ionization reagent gases are being examined because they enhance sensitivity and specificity for analysis of methadone.

ANTIEPILEPTIC DRUGS

B. R. DeMark

The response of patients with epileptic seizures to drug treatment is difficult to predict or assess on the basis of dosages used. A conventional dose may overcontrol or undercontrol seizure frequency, and in the absence of clinical pharmacological information, adjustment of the dose is difficult.

¹Resident Associate, Sankyo Pharmaceutical Company, Tokyo, Japan.

Further, plasma levels of a particular metabolite may be of more consequence than levels of the parent drug. Dr. Dorothy Schottelius (University of Iowa) required the ability to detect and quantitate the drugs and metabolites of phenobarbital, phenytoin, valproic acid, and several other antiepileptic compounds. Derivatization, GC separation, and mass spectrometric identification of these compounds have been carried out. Detection by selected ion monitoring and quantitation by GC-MS-computer techniques have been done on standard mixtures. Patient samples are now available for preliminary exploration of extraction and identification techniques. When these are completed, analysis of samples of patients in clinical studies will be undertaken.

TAURINE KINETIC MEASUREMENTS

C. S. Irving

Taurine (aminoethane sulfonic acid) is a product of cysteine metabolism which has been postulated to be an essential amino acid for the neonate human. In addition to its function in bile acid conjugation, it is a putative neurotransmitter, although this function has not been shown in direct observations. It is an ideal compound for amino acid kinetic measurements, because it is not further metabolized. For clinical studies with Dr. Gerald Gaull, New York Institute for Basic Research on Mental Retardation, it was necessary to develop derivatization, gas chromatographic, and mass spectrometric methods for isotope ratio measurements on taurine. A small quantity of taurine-1,2-¹³C was obtained commercially, and material for clinical studies is being supplied by the Stable Isotope Resource at Los Alamos. Methods were developed for quantitative derivatization and for GC-MS analysis of taurine. A characteristic of taurine derivatives is that they give no response in the flame ionization detector used for gas chromatography. Hence, derivatized taurine can only be recognized and studied by GC-MS. This explains the failure of most analytical methods to quantitate taurine by gas chromatography. When suitable gas chromatographic conditions had been established, isotope ratio measurements were carried out on standard samples. These show that it should be possible to detect taurine at dilutions as low as 1 part in 1000 to 1 part in 3000 in samples of 10 µg. Extraction and purification techniques for taurine in biological samples are being developed.

PYRIDOXINE REQUIREMENTS

B. R. DeMark and J. C. Blais¹

Dr. Steven Coburn (Fort Wayne State Hospital) has sought to establish methods for determining nutritional requirements of the vitamin pyridoxine in man, using pyridoxines labeled with stable isotopes. Synthesis of stable

¹Resident Associate, Institut du Radium, Paris, France.

isotope-labeled forms appears feasible, and we have established the gas chromatography methodology and obtained mass spectra of the various metabolites of pyridoxines. Isotopic abundance measurements on the unlabeled material have been carried out in order to estimate the dilution that can be detected with single or multiple forms of the vitamin. Application of these techniques, particularly to patients with Down's syndrome, will hinge on development of suitable extraction and recovery procedures from urine and plasma. This development is being undertaken by Dr. Coburn; when completed, it will be part of a joint collaboration in the study of pyridoxine kinetics in normal and disease states.

CARBOHYDRATE MALABSORPTION

H. C. Niu

Assessment of carbohydrate malabsorption from the intestine, a disorder that may have serious consequences on the patient's nutritional status, is now possible through very precise measurements of breath hydrogen. In association with Dr. Alfred Baker (Department of Medicine, The University of Chicago), we have refined previous gas chromatographic procedures for separation of hydrogen from other breath constituents and improved its quantitation to the point where hydrogen can be detected in concentrations as low as 2 parts per million in expired air. It can be quantitated at levels of 5 ppm; at levels of 120 ppm, the precision is 1%. It is possible to collect breath samples for subsequent analysis using Vacutainers in an out-patient clinic. Techniques for collecting breath samples have been investigated and it has been found that the absolute levels of hydrogen fluctuate with the conditions of exhalation. However, the internal comparison of breath hydrogen to breath CO₂ levels has shown a constant ratio; so that standardizing the reporting of breath hydrogen values against a constant CO₂ concentration will significantly reduce the variation.

CLINICAL STUDIES

The Argonne Bioanalytical Center continues to occupy a central position in the study of bile acid kinetics. The capability to provide precise kinetic measurements utilizing deuterium- and ¹³C-labeled bile acids and advanced GC-MS-peak switching techniques has attracted clinicians across the country. Current collaborative projects are described in the following reports.

GALLSTONE FORMATION

P. A. Szczepanik-Van Leeuwen and B. R. DeMark

Two separate studies aim to establish the factors that increase the risk of gallstone formation in women. In previous collaboration with Drs. Alan Hofmann and Peter Ng, then at Mayo Clinic, we have examined the bile acid kinetics in several pregnant women from the fifth to eighth week of pregnancy and in healthy, nonpregnant women with and without contraceptive steroid administration. The results of this study indicated that bile acid composition is drastically altered during pregnancy.

These studies have been expanded under the collaboration of Dr. Fred Kern, Jr., University of Colorado Medical Center, to measure duodenal bile acids during all three trimesters of pregnancy, and again after the bile acid pool has returned to normal. The data obtained from this study are yielding unique information about the role of sex hormones in the formation of lithogenic bile and subsequent gallstone formation.

STUDIES OF BILE ACIDS IN LIVER DISEASE

P. A. Szczepanik-Van Leeuwen and F. Stellaard

The identification of the bile acid constituents of the serum, bile, urine, and stool of patients with liver disease has increased in importance with the continuing development of newer GC-mass spectrometric-computer methods for their analysis. We have recently shown that atypical bile acids can be readily identified with great sensitivity using chemical ionization-selected ion monitoring techniques. Therefore, we are in a position to provide the analytical expertise to a number of clinical collaborators who wish to apply this methodology to the study and diagnosis of liver disease.

Of prime importance have been two investigations of children with Zellweger's Syndrome in collaboration with Dr. Russell Hanson, University of Minnesota, and Dr. John Watkins, Harvard Children's Hospital. Analysis of the biological fluids from children with this disease has uncovered evidence of several C-27 bile acids—trihydroxycoprostanic, dihydroxycoprostanic, and varanic acids. The presence of these compounds, normally found only in reptiles and lower animal forms, along with the detection of abnormal mitochondrial structure in these patients, suggests that mitochondrial oxidation is necessary for the formation of C-24 bile acids (chenodeoxycholic and cholic acids) and indicates that a defect in the mitochondrial function may be responsible for the accumulation of C-27 bile acids in Zellweger's Disease.

Byler's disease, a genetic cholestatic liver disease which can be traced to descendants of Jacob Byler, is under investigation in collaboration with Dr. Charles Huang, Baylor Medical College, and with Dr. John Watkins.

Dr. Huang has examined bile levels in serum and urine samples from a child with this type of intrahepatic cholestasis, and compared these samples with two other children with a Byler's-like cholestasis. Hyocholic acid, β -muri-cholic acid, and four compounds thought to be tetrahydroxy-bile acids were identified in the bile, serum, and urine of this child from samples taken over a period of several years. The atypical bile acids detected do not appear to be formed as a result of any drug therapy given this patient.

SYNTHETIC DIETS

P. A. Szczepanik-Van Leeuwen and C. S. Irving

A collaborative project with Drs. Nels R ih , Helsinki, Gerald Gaull, New York Institute for Basic Research on Mental Retardation, and John Watkins, Harvard Children's Hospital, to evaluate the effect of synthetic diets on the growth and development of newborn infants is under way. The pool sizes and fractional turnover rates of chenodeoxycholic and cholic acids are being determined in infants during the first week of life and again after 2 to 3 weeks on a prescribed feeding regime. A total of 20 infants will be studied: 5 on pooled mothers' milk, 5 on a synthetic 60:40 whey:casein diet, 5 on the synthetic diet plus taurine, and 5 on the synthetic diet supplemented with taurine plus cholesterol. Because taurine is essential for infant development, this study will seek to determine whether differences in development can be detected between groups fed mothers' milk and synthetic formulas and, secondly, whether taurine and/or taurine plus cholesterol affect the synthesis and interluminal concentration of bile acids.

METHADONE STUDIES

D. L. Hachey and K. Nakamura¹

Methadone pharmacokinetic studies are continuing in collaboration with Dr. Mary Jeanne Kreek at Rockefeller University. We have completed our single isotope studies of urinary excretion of R-(-) and S-(+)-methadone. We have begun concentrating our efforts on plasma drug level pharmacokinetics using multiple isotopic tracers. Such studies have been hindered in the past because of contamination that interfered with isotope ratio analysis. The problem has been largely overcome and samples are being prepared for analysis. We have completed one multiple isotope study. Cumulative urinary drug excretion data and rate data are consistent with our earlier finding that the two enantiomers of methadone are metabolized differently. It appears that

¹Resident Associate, Sankyo Pharmaceutical Company, Tokyo, Japan.

isotope ratio data provide a much more consistent indication of the drug metabolism than does measurement of the amount of drug excreted or measurement of plasma drug levels.

BREATH TESTS AND FECAL COMBUSTION STUDIES

P. D. Klein and D. A. Schoeller¹

Evaluation of three ^{13}C breath tests employing ^{13}C -labeled fats have been completed in collaboration with Dr. John Watkins and Dr. Barbara Kirschner (The University of Chicago). ^{13}C -labeled triolein, trioctanoin, and palmitic acid were given on successive days to 6 children with an intestinal mucosal disorder, 5 with an intraluminal phase disorder, 5 with a pancreatic disorder, and 4 normal children. The triolein breath test clearly identified the 16 abnormalities, demonstrating it to be a superior screening test for fat malabsorption. The palmitic acid breath test, in combination with the triolein breath test, was specific for pancreatic disorders and may be a noninvasive alternative to the pancreatic stimulation test which requires intubation. The trioctanoin breath test was the least specific, giving poor separation of children with mucosal or intraluminal disorders from normals.

A 2-year longitudinal study of liver function in patients after jejunal/ileal bypass surgery for the treatment of morbid obesity has begun in collaboration with Drs. John Schneider and Alfred Baker (The University of Chicago). The aminopyrine breath test will be performed along with the standard liver function tests before and at 3, 6, 12, 18, and 24 months after bypass surgery. Fifteen subjects have entered the protocol. Of these, one has developed hepatic complications that occur in about 10% of bypass patients. The presurgery aminopyrine breath test of the subject who developed hepatic complications was lower than the 14 who did not, and breath tests showed a further loss of liver function before the other liver tests indicated hepatic complication. Further subjects will be included in the protocol to see whether the breath test continues to identify those subjects at risk.

A fecal assay for the detection of unabsorbed bile acids has been developed and applied to the detection of bile acid malabsorption in eight children in collaboration with Dr. John Watkins. Feces are lyophilized and combusted to CO_2 , and the excess ^{13}C from the labeled bile acid is detected by isotope ratio analysis. The test can detect less than 1% bile acid malabsorption. The fecal test is used in conjunction with a breath $^{13}\text{CO}_2$ test to detect intestinal bile acid deconjugation. Neither the breath test nor fecal test alone can be used for the diagnosis of intestinal bile salt disorders.

¹Resident Associate, Division of Biological and Medical Research, and Research Associate (Assistant Professor), The University of Chicago.

INFORMATION DISSEMINATION AND TRAINING

P. D. Klein, E. R. Klein,¹ D. A. Schoeller,² and D. L. Hachey

The Third International Conference on Stable Isotopes was held May 23-26, 1978 at Oak Brook, Illinois. There was a total of 190 attendees representing industry and academic institutions from the United States and many European countries, including East Germany, as well as Japan and Australia. Of the 37 attendees representing industry, 10 were exhibitors; of the 153 attendees representing academic institutions, 20 were graduate students whose attendance was supported by a generous grant from the Argonne Universities Association. Seventy papers were given during the conference which dealt with environmental, clinical, pharmacological, biochemical, and methodological applications of stable isotopes. A poster session was held covering the same general range of topics. The editing of the papers presented is nearing completion, and the papers will be published by Academic Press as "Stable Isotopes: Proceedings of the Third International Conference." The publication will appear in 1979.

There appears to be a demand for an update of the selected bibliographies of biomedical and environmental applications of ^2H , ^{13}C , ^{15}N , ^{17}O , ^{18}O , and ^{34}S . The data base used previously and the response from readers indicate that every 2 years there is an increase of 50% in the numbers of publications dealing with stable isotopes. This number may be even greater for papers dealing with multiple isotope usage in environmental applications. Our update will emphasize the increase in environmental applications and will cover 1976-1978. As before, the references will be indexed by author and subject to increase utility.

Ms. Makiko Suehiro, of the Tokyo Metropolitan Geriatric Hospital, Department of Nuclear Medicine, completed her year as a Visiting Scientist and has returned to establish a Laboratory of Stable Isotopes. We are continuing to maintain advisory liaison in the selection and installation of isotope ratio equipment. Two Visiting Scientists are spending FY 1979 at the Argonne Bioanalytical Center, under the sponsorship of their home institutions, to obtain training and experience in the application of stable isotopes and GC-MS to clinical and biochemical problems. These individuals are Dr. Kanichi Nakamura from Sankyo Pharmaceutical Company, Tokyo, Japan, and Dr. Jean-Claude Blais from the Institut du Radium, Paris, France. Continued exchange with the Japan Stable Isotope Committee and with the Japanese Society for Medical Mass Spectrometry was fostered by their sponsorship of a tour by Peter Klein during November, 1978.

¹Resident Associate, Division of Biological and Medical Research.

²Resident Associate, Division of Biological and Medical Research, and Research Associate (Assistant Professor), The University of Chicago.

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19. SUPPORT FACILITIES

ANIMAL FACILITIES

James G. Linsley, Thomas E. Fritz, Patricia C. Brennan, William G. Keenan, Calvin M. Poole, Richard C. Simkins, and David V. Tolle

James G. Linsley assumed the operational responsibilities of the Animal Facilities when he joined the Division as Manager of Animal Facilities on June 1, 1978. Dr. T. E. Fritz returned to full-time research, but remains as Chairman of Animal Health and Clinical Services Committee.

The Laboratory Animal Facilities, including the central area in Building 202 and the satellite facility specifically utilized for viral oncology in Building 340, comprise approximately 33.8% (39,532 square feet) of the total usable space in the Division.

The closed breeding colony of beagles utilizes 9,630 square feet. Six kennels contain 380 runs and housed an average of 750 dogs in 1978. Each run consists of a heated indoor compartment plus an outdoor exercise area. Technical equipment area allocated for the support of the dog programs occupies another 8,230 square feet. This includes three gamma exposure rooms, two dog treatment rooms, an operating room, necropsy room, and X-ray room. The remaining 45% (17,860 square feet) of the Animal Facilities consists of 47 animal rooms and rooms for cage and bottle washing, sterilization, and injection of radioactive materials. Each of the animal rooms is capable of holding laboratory animals under controlled conditions of temperature, humidity, and lighting.

During 1978, approximately 26 staff members of the Division were involved in various studies utilizing in excess of 30,000 rodents and other laboratory animal species.

The supporting facilities for the husbandry and clinical monitoring of the resident animals and the responsible personnel include:

- 1) Automated cage and bottle washing and filling machinery (Animal Care Specialists)
- 2) Steam and gas autoclaves for sterilization of equipment, instruments, and media
- 3) Diagnostic X-ray facilities and darkrooms (C. M. Poole, W. G. Keenan)
- 4) Clinical pathology laboratory (D. V. Tolle, R. C. Simkins)

- 5) Hematology Laboratory (D. V. Tolle)
- 6) Diagnostic microbiology laboratory (R. C. Simkins, P. C. Brennan)
- 7) Necropsy laboratory (T. E. Fritz)
- 8) Surgical suite with inhalation anesthesiology equipment (C. M. Poole, W. G. Keenan)
- 9) Gnotobiotic isolators for germ-free technology (J. G. Linsley, L. O. Bibbs)

Funds have been allocated for the renovation and modernization of the kennels, the engineering and design work has been completed, and work is scheduled to begin in the spring of 1979. The renovations will remove the fiberglass tub liners and provide new run dividers, gutters, doors, and gates. The design of the new dividers and gates will enable use of automated cleaning equipment and will result in more efficient cleaning procedures and better sanitation. In addition, renovations to E wing have been largely completed and this new facility will be reoccupied early in the next year. This facility includes 26 animal holding and breeding rooms for rodents and other small laboratory animals. The renovations included entirely new light, heating, cooling, and humidity control and air filtration equipment.

An important aspect of the success of any animal research program is the health of its animal population. To assure a supply of acceptable high quality animals, the Facility has concentrated on breeding its own healthy, disease-free animals. Most of the rodents, particularly mice, and all dogs are bred in the Facility. The rodent breeding is managed by LeRoy O. Bibbs and Jane M. Angerman. The beagle breeding is supervised by Calvin M. Poole and William G. Keenan, who are also responsible for the clinical and surgical care of the dogs. The beagle colony has been a closed colony for more than 19 years, and extensive computerized records are maintained on all aspects of the colony, including reproduction, genetics, hematology, pathology, and disease incidence.

As important as the physical plant and its support facilities are the staff available to manage, monitor, treat, and evaluate the animals. The personnel provide a complete range of services to users of the experimental animals. Care of the animals is performed by a group of Animal Care Specialists, listed below, under the supervision of Durward D. Banister and William H. Hart.

The Animal Care Specialists during 1978 were the following:

Earl R. Allen
 Roberta R. Buller
 Mose Burrell
 Carl C. Colegrove¹
 Claude C. Colegrove¹

¹Group Leader

Lucille E. Daley
 Leo C. Farcus¹
 Charles J. Fowler
 Carrey R. Herringer
 Edward W. Jackson¹
 James L. Johns
 William G. McDade, Jr.¹
 Ann L. Mize
 Kenneth R. Muller
 Cathleen L. Nelson
 William O. Robinette
 Bernard A. Royer
 Richard M. Santarelli
 Susan L. Santarelli
 Leon L. Stewart
 Diane M. Thomas
 Rudolph H. Tiedt
 Joseph N. Wilson

COMPUTER SUPPORT FACILITIES

Frank S. Williamson, Jeanne A. Blomquist, and Carol A. Fox

Computer support is centered on the Remote Access Data Station (RADS), which is equipped with a 1000 lpm printer, 1000 cpm reader, and a 300 cps paper tape reader with 500-foot spools. The RADS is located in a data preparation room with four 029 key punches (two of which interpret), a storage vault for archival magnetic tapes, card files, and a 30 cps interactive terminal principally used for job inquiry and routing. An adjacent room provides work space for users, with a documentation library and a consultant's office, plus file storage for programs and their documentations.

Approximately 50 users in the Division continue to depend on these facilities and the consultation on job management, programming, and data management that we provide.

ELECTRON MICROSCOPE CENTER

Thomas M. Seed and G. Theodore Chubb

The facility has approximately 2,600 square feet of working laboratory space, and includes two fully equipped photographic darkrooms, sectioning and autoradiographic facilities, six microscope cubicles, and five transmission

¹Group Leader

electron microscopes and one Cambridge scanning electron microscope equipped with an X-ray energy dispersive analytical system. Ancillary specimen preparative equipment includes vacuum evaporators, freeze-drying and freeze-etching equipment, ultramicrotomes, and assorted photographic and light microscopic equipment.

The Electron Microscope Center and its personnel provide specialized electron microscope services to both the Division and the Laboratory as a whole. G. T. Chubb continues to maintain EM-related equipment and to provide needed technical assistance to all users of the Center. Active collaborative interaction between the Center and various Divisional research groups continues (e.g., the Cellular Indicators Program; see Section 3). Ultrastructural data were presented at various local, national, and international meetings (e.g., 9th International Congress for Electron Microscopy, Toronto, Canada, 1978). In addition, the Center has, through the active support of Argonne's Center for Educational Affairs and with Western Michigan University, established a technical training program for postgraduate individuals interested in pursuing a career in electron microscopy.

During 1978 there were some 25 researchers who made active use of the available facilities. They included 11 regular staff members, 2 postdoctoral appointees, 3 graduate students, and 4 visiting scientists. The remainder of the users were from other Argonne divisions, including Radiological and Environmental Research, Environmental Impact Studies, Chemistry, Physics, and Solid State Science.

20. EDUCATIONAL ACTIVITIES

POSTGRADUATE TRAINING

During 1978, a total of 32 postdoctoral appointees and research associates contributed to the research programs of the Division. Fifteen of these were new appointments in 1978, one less than the number who finished their assignments during the year.

The temporary appointees, their schools, and the staff members with whom they were affiliated were as follows:

N. Leigh Anderson ¹	Cambridge University, England	D. Grahn
Nadine N. Beales	University of Illinois at the Medical Center	C. Peraino
Laszlo Bodoni	Loyola University, Chicago	C. A. Reilly
Donna M. Buchholz	University of Illinois at the Medical Center	T. M. Seed
Anne L. Cahill	University of Michigan, Ann Arbor	C. F. Ehret
Bruce R. DeMark	University of Southern California	P. D. Klein
Jesse J. Edwards	University of Alabama, Birmingham	N. G. Anderson
Karen B. Ekelman	Ohio State University	C. Peraino
Michael E. Ginevan	University of Kansas	D. Grahn
Michael N. Gould	University of Wisconsin, Madison	M. M. Elkind
Viola M. Griego	Washington State University	R. B. Webb
Wayne R. Hanson	University of Iowa	C. A. Reilly
Nelson D. Horseman	Louisiana State University, Baton Rouge	C. F. Ehret
Charles S. Irving	Weizmann Institute, Israel	P. D. Klein
Frederick R. Kirchner	University of Iowa	P. C. Brennan
George R. Lankas	University of Cincinnati	M. M. Elkind
Chung Hee Ryu Lee ²	University of Illinois, Urbana	D. Grahn

¹Research Associate, now Assistant Biophysicist.

²Research Associate.

Joel R. Mitchen ¹	Life Sciences, Incorporated, St. Petersburg	C. A. Reilly
Chester N. Newman	Indiana University Medical Center, Indianapolis	H. E. Kubitschek
Hsien-Chi Niu	University of Missouri, Kansas City	P. D. Klein
Kanaiyalal R. Patel	University of South Dakota	Y. E. Rahman
Meyrick J. Peak ¹	Rhodes University, South Africa	H. E. Kubitschek
Pepi Ross-Riveros	University of California, Berkeley	M. M. Elkind
Eung K. Ryu	Michigan Cancer Foundation, Detroit	M. MacCoss
Herbert M. Schwartz	Brandeis University	S. S. Danyluk
Carol F. Smith	University of Illinois at the Medical Center	N. G. Anderson
Elsie M. Sorensen	University of Texas, Austin	A. Lindenbaum
Frans Stellaard ¹	University of Technology, The Netherlands	P. D. Klein
Fred J. Stevens	Michigan State University, East Lansing	M. Schiffer
Ravindra Tewari	Tata Institute of Fundamental Research, Bombay, India	S. S. Danyluk
Kou-Yi Tserng	University of Michigan	P. D. Klein
Hiroshi Utsumi	Kyoto University, Japan	M. M. Elkind

In addition, there were nine Faculty Research Participation appointments, supported by the Argonne Center for Educational Affairs (CEA) and four Visiting Scientists; these appointments enable college and university faculty members to participate in the research activities of the Laboratory in order to broaden their perspectives for teaching and research on their home campuses. The names of the Faculty Research Participants and Visiting Scientists during 1978, their schools, and their staff sponsors were as follows:

Jay B. Best	Colorado State University	G. A. Sacher
Frederic A. Gliere	Lake Forest College	N. G. Anderson
Ronald D. Humphrey	Prairie View A&M University	C. A. Reilly
Margaret M. Jonah	Rosary College	Y. E. Rahman
Ellen H. Lau	College of DuPage	Y. E. Rahman
Eugene W. McArdle	Northeastern Illinois University	C. F. Ehret
John P. Neenan	Illinois Benedictine College	M. MacCoss
Daniel G. Oldfield	DePaul University	T. M. Seed
Nicolas Panagiotopoulos ²	Nuclear Research Center, Athens, Greece	M. Schiffer
Ramaswamy H. Sarma ²	State University of New York at Albany	S. S. Danyluk
John R. Seed ²	Texas A&M University	T. M. Seed

¹Research Associate.

²Visiting Scientist.

S. Peter Spragg ¹	University of Birmingham, Birmingham, England	N. G. Anderson
Timothy A. Stabler	Indiana University Northwest, Gary	M. M. Elkind

SUMMER RESEARCH INSTITUTE IN CELL BIOLOGY

Eleven students from nine different universities were enrolled during the Summer of 1978 in this graduate level program offered by the Division of Biological and Medical Research in cooperation with the Argonne Center for Educational Affairs. Dr. Mortimer M. Elkind served as organizer and co-ordinator, with the assistance of M. McCoy. The program, which ran for 12 weeks, featured a lecture series covering a variety of topics, including lethality and mutagenesis, DNA repair, neoplastic transformation, radiation and the cardiovascular and hematopoietic systems, responses to effluents of coal combustion, metabolism of chemical carcinogens, and mechanisms of therapy of metal poisoning. The lectures were given by Drs. M. H. Bhattacharyya, P. C. Brennan, E. Chan, A. Han, D. A. Haugen, T. Matsushita, Y. E. Rahman, C. A. Reilly, T. M. Seed, S. P. Stearner, and R. B. Webb.

The lectures were supplemented by informal discussions and visits to laboratories as appropriate. Each student spent the remainder of his time working in a laboratory of a staff member.

The students, their schools, and their staff sponsors were as follows:

Barbara A. Amsler	University of Wisconsin, Milwaukee	C. A. Reilly
Terry M. Bricker	Miami University	Y. E. Rahman
Mary A. Gabriel	University of Dallas	T. E. Fritz
James Hardwick	Illinois State University	D. A. Haugen
Louis M. Lissuzzo	Loyola University, Chicago	P. C. Brennan
Dawn Palter	University of Texas, Austin	T. M. Seed
Terry Riss	Illinois State University	S. P. Stearner
Kevin Rupprecht	University of Notre Dame	E. Chan
Ellen Saltz	University of Wisconsin, Whitewater	M. Bhattacharyya
David Simpson	University of Illinois, Urbana	A. Han
Albert Sorrell	State University of New York at Plattsburgh	T. Matsushita

OTHER GRADUATE PROGRAMS

Four graduate students were Laboratory Graduate Participants working in the Division on research for their PhD degrees in a program administered and supported by the Center for Educational Affairs. The Laboratory Graduate Participants, their schools, and their staff sponsors were as follows:

¹Visiting Scientist.

Thomas H. Cunningham	University of Illinois at the Medical Center	D. Grahn
Steven H. Gray	University of Illinois at the Medical Center	M. MacCoss
Michael P. Hagan	University of Illinois, Urbana	M. M. Elkind
Bruce Hammer	Northwestern University	S. S. Danyluk

A related program, called Thesis Parts, is supported by the Center for Educational Affairs. It enables graduate students to perform pertinent parts of their research at Argonne. In 1978, two students held such appointments in the Division:

Dawn Palter	University of Texas, Austin	T. M. Seed
Roger G. Ulrich	Western Michigan University	T. M. Seed

In addition, six graduate students carried out research in the Division as Resident Student Associates, supported directly by the Division unless otherwise noted.

Michael D. Brown ¹	University of Illinois at the Medical Center	T. E. Fritz
James J. Collins	University of Illinois, Urbana	D. Grahn
Hyojoon Hahn ²	Medical University of South Carolina	N. G. Anderson
Paul Maffuid	St. Michael's College	S. S. Danyluk
William Obermeyer	University of Chicago	G. A. Sacher
Karen E. Willard	Virginia Polytechnic Institute and State University	T. E. O'Connor/ N. G. Anderson

UNDERGRADUATE TRAINING

During 1978, a total of 37 college undergraduates received training in the Division of Biological and Medical Research through the CEA-sponsored Spring, Summer, and Fall Undergraduate Research Participation Programs. The students, their schools, and their staff supervisors are listed below:

SPRING PROGRAM

John M. Fritz	University of Kentucky	B. S. Hass
Mary A. Gabriel	University of Dallas	T. E. Fritz
Alan H. Gorn	University of Chicago	P. D. Klein
Jennie R. Hahn	College of St. Francis	B. S. Hass
Jeffrey Holmgren	University of Wisconsin, River Falls	B. S. Hass
JoAnn Johnston	College of St. Francis	D. L. Hachey
Gregory Leo	West Chester State College	M. MacCoss
Tami Levitt	Oberlin College	C. F. Ehret
Elaine M. Mader	The Benedictine College	B. S. Hass

¹Sponsored by the Argonne Affirmative Action Program.

²Sponsored by Abbott Laboratories.

Martha J. Monnett	University of North Carolina at Greensboro	B. S. Hass
Maureen Schilb	College of St. Francis	P. D. Klein
Marcia L. Zucker	Rensselaer Polytechnic Institute	C. A. Reilly

SUMMER PROGRAM

Lisa Antonelli	Southeastern Massachusetts University	C. F. Ehret
Helen C. Burke	University of San Diego	C. F. Ehret
Gary Duckwiler	Claremont Men's College	M. MacCoss
John M. Fritz	University of Kentucky	B. S. Hass
Jerald Hendrix	Shorter College	M. Schiffer
Steven Holland	St. John's College, Annapolis	T. E. O'Connor
Jeffrey Holmgren	University of Wisconsin, River Falls	B. S. Hass
Robert Killeen	Northern Illinois University	C. F. Ehret
Sunny Kim	Northwestern University	S. S. Danyluk
Lai-Yet Lam	Dickinson College	T. M. Seed
Tami A. Levitt	Oberlin College	C. F. Ehret
Edward Lewandowski	University of Chicago	T. E. Fritz
Doriane C. Miller	Illinois Institute of Technology	T. E. O'Connor
James R. Milstead	Southern Illinois University	D. A. Haugen
Kimberly Nortridge	Northern Illinois University	M. P. Finkel
Rachel Pocinki	Swarthmore College	C. F. Ehret
Anita Rae	University of Portland	C. A. Reilly
Linda Ross	Rochester Institute of Technology	N. G. Anderson
Michael Skinner	Georgia Institute of Technology	G. A. Sacher
Richard S. Skyer	Rochester Institute of Technology	S. P. Stearner
Aurelia V. Smith	Mississippi Valley State University	M. M. Elkind
Paul Underwood	Morehouse College	C. Peraino
Luz Venta	Northwestern University	H. E. Kubitschek
Craig Zabel	Northern Illinois University	Y. E. Rahman

FALL PROGRAM

Wendell Bauman	Nebraska Wesleyan University	D. A. Haugen
Emily Brooks	Harding College	B. S. Hass
Robert Last	Ohio Wesleyan University	M. MacCoss
Randall Updegrove	University of Rhode Island	C. A. Reilly

JOINT ARGONNE-UNIVERSITY APPOINTMENTS

During 1978, 19 staff members held a total of 28 faculty appointments at universities in the Chicago area. These appointments usually comprise limited teaching activities at the graduate level, generally of a specialized nature,

which involve regular contact with students. They have led to cosponsorship of graduate students and to collaborative research efforts with faculty members, some of which are described in this report.

The affiliations with Chicago area universities were as follows:

University of Chicago

Mortimer M. Elkind	Timothy E. O'Connor
Robert N. Feinstein	George A. Sacher
David L. Hachey	Warren K. Sinclair
Peter D. Klein	Patricia Szczepanik-Van Leeuwen

University of Illinois at Chicago Circle

Douglas Grahn	Warren K. Sinclair
Herbert E. Kubitschek	John F. Thomson
Carl Peraino	

Loyola University

Thomas E. Fritz	Arthur Lindenbaum
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Northern Illinois University

Maryka H. Bhattacharyya	Yueh-Erh Rahman
Thomas E. Fritz	Christopher A. Reilly, Jr.
Douglas Grahn	Warren K. Sinclair
Herbert E. Kubitschek	John F. Thomson
Carl Peraino	Robert B. Webb

Northwestern University School of Medicine

Peter D. Klein

University of Health Sciences/Chicago Medical School

Antun Han

University of Illinois at the Medical Center

Steven S. Danyluk

21. DIVISIONAL SEMINARS DURING 1978

During the first half of 1978, the Division of Biological and Medical Research Seminar Committee consisted of M. H. Bhattacharyya, D. L. Hachey, A. Han, M. MacCoss, D. V. Tolle, and T. M. Seed, Chairman. For the 1978-1979 seminars, the Committee consisted of L. S. Lombard, R. T. Lundy, C. Peraino, T. M. Seed, M. MacCoss, Vice Chairman, and A. Han, Chairman.

In addition to the regular Thursday afternoon seminars by Argonne and visiting scientists, the 1977-1978 Committee initiated a Distinguished ANL Scientist Lecture Series in which Argonne researchers who have made important scientific contributions over the years in their respective fields of biology were invited to address the Divisional staff and the Laboratory at large to review the evolution in their fields, the speaker's own contributions, and their speculations on the future of their areas. The first speaker in the series was Joseph J. Katz, Chemistry Division, who spoke on September 29, 1977, on "Chlorophyll Function in Photosynthesis." In 1978, the second lecture in the series was given by George A. Sacher on March 9, and was entitled "Energy and Life: The Relation of Longevity to Metabolic Rate and Body Size in Mice." The third was presented by Miriam P. Finkel on June 29, and was entitled "Experimental Induction of Bone Tumors."

The Seminar Program for 1978, selected by the Committee on the basis of recommendations from the staff, was as follows:

Dr. Thomas M. Seed, Division of Biological and Medical Research, Argonne National Laboratory

"Hemopathological Consequences of Protracted Gamma Irradiation: Preclinical Phases of Leukemia Induction"

January 3, 1978

Dr. Gerald C. Mueller, McArdle Laboratory for Cancer Research, Madison

"DNA and Chromatin Replication--Possible Coupling in Cell Differentiation"

January 5, 1978

Dr. D. Fleming, Atlantic Richfield Company, Harvey, IL

"Energy for the Future--A Research Appraisal"

January 10, 1978

Dr. Gordon Niswender, Colorado State University, Fort Collins

"Endocrine Regulation of Ovarian Function"

January 12, 1978

Dr. Wayne R. Hanson, Division of Biological and Medical Research, Argonne National Laboratory

"Effects of High Specific Activity $^3\text{HTdR}$ and Hydroxyurea in Combination with Colcemid and Radiation on Intestinal Stem Cells"

January 17, 1978

Dr. Garth Nicolson, University of California, Irvine

"Cellular Interactions during Blood-borne Tumor Metastasis"

January 19, 1978

Dr. Robert B. Webb, Division of Biological and Medical Research, Argonne National Laboratory

"Lethal and Mutagenic Effects of Solar Ultraviolet Radiation"

January 31, 1978

Dr. Alphonse E. Sirica, University of Wisconsin, Madison

"Primary Cultures of Adult Rat Hepatocytes on Floating Nylon Meshes Coated with a Thin Collagen Matrix: Expression of Fetal Liver Cell Properties"

February 2, 1978

Drs. Christopher A. Reilly, Jr., Miriam P. Finkel, and Emerson Chan, Division of Biological and Medical Research, Argonne National Laboratory

"Role of Retroviruses in Radiation Tumorigenesis"

February 7, 1978

Dr. Alan Jeffrey, Columbia University

"Benzo(a)pyrene Nucleic Acid Interactions"

February 9, 1978

Dr. Kenneth Wilzbach, Chemistry Division, Argonne National Laboratory

"Environmental Control Technology--Power Generation Program"

February 14, 1978

Prof. Arthur D. Broom, University of Utah

"Polyribonucleotides Containing Thiopurines: Synthesis, Characterization, and Biological Activity"

February 16, 1978

Drs. Dale A. Schoeller, David L. Hachey, Kou-Yi Tserng, and Peter D. Klein, Division of Biological and Medical Research, Argonne National Laboratory

"Being Useful to Clinicians: Methods in Our Madness"

February 21, 1978

Dr. P. Nelson, Chemical Engineering Division, Argonne National Laboratory

"The Future of Electric Vehicles"

February 28, 1978

Dr. James N. Ihle, Frederick Cancer Research Center, Maryland

"The Role of C-Type Virus in Murine Leukemias"

March 2, 1978

Drs. David A. Haugen and Carl Peraino, Division of Biological and Medical Research, Argonne National Laboratory

"Carcinogens: Identification and Mechanisms of Action (A Report of the 31st Annual M. D. Anderson Hospital Symposium)"

March 7, 1978

Mr. George A. Sacher, Division of Biological and Medical Research, Argonne National Laboratory

"Energy and Life: The Relation of Longevity to Metabolic Rate and Body Size in Mice"

March 9, 1978

Dr. R. G. Matlock, Office of the Director, Argonne National Laboratory

"ANL Solar Programs"

March 14, 1978

Prof. Elwood V. Jensen, Ben May Laboratory, Chicago

"Hormone Dependency in Mammalian Tissues and Tumors"

March 16, 1978

Dr. Michael J. Dewey, The Institute for Cancer Research, Philadelphia

"Mosaic Mice Produced from Mutant Teratocarcinoma Cells"

March 23, 1978

Dr. Peter C. Uden, University of Massachusetts, Amherst

"Modern Peak Identification Methods for Gas and High Pressure Liquid Chromatography"

March 30, 1978

Dr. Malcolm MacCoss, Mr. Paul Maffuid, and Dr. Ravindra Tewari, Division of Biological and Medical Research, Argonne National Laboratory

"Aspects of Molecular Studies Research"

April 4, 1978

Dr. Janardeen K. Reddy, Northwestern University

"Hepatic Peroxisome Proliferation: Role in the Development of Hypolipidemia, Hepatomegaly, and Hepatocellular Carcinoma"

April 6, 1978

Mr. Michael P. Hagan, Division of Biological and Medical Research, Argonne National Laboratory

"Near-Ultraviolet Light Effects on Chinese Hamster Cells Pulse-Labeled with Bromodeoxyuridine"

April 11, 1978

Dr. Gary Van Zant, University of Chicago

"Regulation of Erythroid Differentiation"

April 12, 1978

Dr. G. T. Kartsounes, Energy And Environmental Systems Division, Argonne National Laboratory

"Mechanical Energy Storage: Compressed Air and Underground Pumped Hydro"
April 18, 1978

Prof. Hector DeLuca, University of Wisconsin, Madison

"New Developments in the Metabolism and Function of Vitamin D"
April 24, 1978

Dr. Robert T. Lundy, Division of Biological and Medical Research, Argonne National Laboratory

"Modelling the Effects of Energy-Related Effluents on Human Health or Is Coal Addictive?"
April 25, 1978

Dr. William Campbell, Mayo Clinic

"Isolation and Characterization of a Variant Primate Retrovirus"
April 27, 1978

Dr. C. C. Baker, Energy and Environmental Technology Division, Argonne National Laboratory

"Argonne's Fusion Power Program"
May 2, 1978

Dr. Pamela McAthey, University of Edinburgh, Scotland

"Mutation Studies on Continuous Cultures of the Fission Yeast, Schizosaccharomyces pombe"
May 4, 1978

Dr. James Rodgers, Tufts University Medical School

"Physical Considerations for Localised Hyperthermia with RF Electric Currents"
May 8, 1978 (joint seminar with Radiological and Environmental Research Division)

Dr. Siegmund J. Baum, Defense Nuclear Agency, Bethesda

"Regulation of Hematopoiesis, in Vitro"
May 11, 1978

Dr. Lawrence E. Scheving, University of Arkansas Medical Center

"Chronobiology and Its Applications in Experimental Cancer Chemotherapy"
May 18, 1978

Dr. Jay B. Best, Colorado State University

"Modeling Radiation Responses"
May 24, 1978

Dr. Ruth Ben Ishei, Rutgers University

"DNA Repair in Isolated Human Cell Nuclei"
May 25, 1978

- Dr. R. Schoenborn, Brookhaven National Laboratory
"Low Angle Neutron Scattering of Biological Molecules"
May 26, 1978
- Dr. Ronald E. Davies, Temple University School of Medicine
"Biochemistry of Hydrocarbons and Carcinogenic Effects"
June 1, 1978
- Dr. Eliezer Huberman, Oak Ridge National Laboratory
"Carcinogenesis, in Vitro"
June 8, 1978
- Dr. David R. Woods, Rhodes University, South Africa
"Regulation of Collagenase Production in Achromobacter sp."
June 15, 1978
- Dr. Miriam P. Finkel, Division of Biological and Medical Research, Argonne National Laboratory
"Experimental Induction of Bone Tumors"
June 29, 1978
- Dr. Johann Deisenhofer, Max-Planck Institut, Munich, West Germany
"Three Dimensional Structure of Immunoglobulins"
August 7, 1978
- Dr. G. Zaccai, Institute Max von Laue-Paul Langevin, Grenoble, France
"Applications of Neutrons to Biological Structure Problems"
August 10, 1978
- Dr. Theodor M. Fliedner, University of Ulm, West Germany
"Collection, Storage, and Use of Blood Derived Hemopoietic Stem Cells for Bone Marrow Reconstitution in Lethally Irradiated Beagles"
August 24, 1978
- Dr. R. K. Poole, University of London, England
"Oscillatory Respiration During the Cell Cycle of Yeast in E. coli"
September 14, 1978
- Dr. Tatsuo Matsushita, Division of Biological and Medical Research, Argonne National Laboratory
"DNA Repair and Sister Chromatid Exchanges in Mouse Myeloma Cells"
September 28, 1978
- Dr. Jung-Chung Lin, The University of North Carolina, Chapel Hill
"Biochemical Markers for Premalignant Hepatocytes During Liver Carcinogenesis"
October 5, 1978
- Prof. R. Schulte Hermann, University of Marburg, Lahn, Germany
"Effects of Mitogenic Agents and Nutrition on Growth Control in Normal and Preneoplastic Rat Liver"
October 12, 1978

Prof. M. Sundaralingam, University of Wisconsin, Madison

"Nucleotide Conformations and Their Relationship to t-RNA Structures"

October 19, 1978

Dr. James I. Garrels, Cold Spring Harbor Laboratory, New York

"Differentiation of Culture Nerve and Muscle Cells: A Study by Quantitative Two-Dimensional Gel Electrophoresis"

November 2, 1978

Dr. Robert B. Painter, University of California, San Francisco

"Inhibition of DNA Replicon Initiation by Carcinogens"

November 9, 1978

Dr. Robert O. Poyton, University of Connecticut, Farmington

"Mitochondrial Membrane Biogenesis: Co-ordination of Mitochondrial and Nucleo-Cytoplasmic Genetic Systems in the Assembly of Cytochrome c Oxidase"

November 16, 1978

Dr. Maryka H. Bhattacharyya, Division of Biological and Medical Research, Argonne National Laboratory

"I. A Mechanism for the DTPA-Induced Removal of Hepatically Deposited Plutonium. II. A Brief Perspective on the Battery Assessment Program"

November 30, 1978

Dr. Leo T. Furcht, University of Minnesota Medical School, Minneapolis

"Membrane Immunocytochemical and Structural Changes in Cellular Transformation and Differentiation"

December 7, 1978

Dr. Martin A. Hamilton, Montana State University, Bozeman

"The Detection of Synergistic Effects in Carcinogenesis"

December 14, 1978

In addition, a number of informal seminars in specialized subjects were held during the year. These included the following:

Dr. Robert W. Tuveson, University of Illinois, Urbana

"Evidence for a Gene Controlling Black Light Independent of the recA Gene in the Strain of E. coli K12"

January 11, 1978

Dr. Laszlo Bodoni, Loyola University

"The Interactions of Thioxanthenones with DNA and Their Effects on Template Activity"

January 20, 1978

- Dr. Nadine Beales, University of Illinois at the Medical Center, Chicago
 "Transformation-Defective Viable Deletion Mutant of SV40"
 February 16, 1978
- Dr. Karen Ekelman, The Ohio State University
 "The Effects of Benzo(a)pyrene and 7,12-Dimethylbenz(a)anthracene on Human Fibroblasts in Vitro"
 February 24, 1978
- Dr. Erik Boye, Norwegian Radium Hospital, Norway
 "Repair of DNA Double-Strand Breaks in E. coli"
 March 1, 1978
- Dr. Gary Van Zant, University of Chicago
 "Interactions of Erythropoietin with Responsive Stem Cells"
 March 1, 1978
- Dr. Curtis Graeber, Walter Reed Hospital
 "Effects of Meal Timing on Human Circadian Rhythms"
 March 27, 1978
- Dr. Frederick R. Kirchner, University of Iowa
 "Detection, Partial Characterization and Partial Purification of a Small Intestinal Chalcone"
 March 31, 1978
- Dr. Melissa Woan, University of Illinois, Urbana
 "Dose Dependent Activation of Cytotoxic Lymphocytes by Vaccinia Virus Enhancers"
 March 31, 1978
- Dr. Robert M. Sklar, University of Chicago
 "Linked Mutation Induction"
 April 4, 1978
- Dr. Neena B. Schwartz, Northwestern University
 "A New Non-Steroid Hormone from the Ovary: Folliculostatin"
 April 7, 1978
- Dr. Nelson D. Horseman, Louisiana State University, Baton Rouge
 "Temporal Hormone Synergisms: Consequences and Possible Mechanisms"
 April 3, 1978
- Dr. Byung-Moo Soh, Illinois Institute of Technology
 "Control of Glutamine Synthetase mRNA by Hydrocortisone during Embryonic Development of Chick Retina"
 April 28, 1978
- Drs. James Lipscomb and Michael Pique, University of North Carolina, Chapel Hill
 "Computer Graphics and Crystallography"
 May 1-3, 1978

- Ms. Viola Griego, Washington State University
"Properties of the Insecticidal Bacterium Bacillus thuringiensis"
May 5, 1978
- Dr. Kenneth P. Samuel, Georgetown University
"DNA-Nickase Associated with Avian Myeloblastosis"
May 19, 1978
- Dr. Charles Borso, Princeton University
"New Techniques in Structure Determination of Macromolecules Using X-Ray and Neutron Scattering"
May 31, 1978
- Dr. Thomas W. Bednar, Michael Reese Medical Center, Chicago
"Chemical Carcinogenesis: A Peroxidative Activation Mechanism"
June 15, 1978
- Dr. John W. Bornhoeft III, Loyola University Medical Center, Maywood
"Lytic Cycle Replicative Intermediates of Prophage P₁ and P₁ Dlac"
June 28, 1978
- Dr. Ronald Watson, Purdue University
"The Secretory Immune System of the Dog"
July 14, 1978
- Dr. Robert Heintz, State University of New York, Plattsburgh
"Ribosomal GTP Hydrolysis"
August 14, 1978
- Dr. Michael Rush, Florida State University, Tallahassee
"Inhibin--The Newest Gonadal Hormone"
September 20, 1978
- Dr. Marius Teodorescu, University of Illinois Medical Center, Chicago
"Use of Bacteria for the Identification of Functionally Different Lymphocyte Subpopulations in Blood Smears"
December 12, 1978

22. OUTSIDE TALKS BY DIVISIONAL STAFF DURING 1978

- Anderson, N. G. "The Molecular Anatomy and Characterization of Tissue Proteins." Department of Human Oncology, Wisconsin Clinical Cancer Center, University of Wisconsin, Madison, WI, January 17, 1978.
- Anderson, N. L. "High Resolution 2-D Electrophoresis of Human Hair Follicles and Lymphocytes: Application to Genetic Screening." Joint Biophysical Society-American Physical Society Meeting, Washington, DC, March 27-30, 1978.
- Bhattacharyya, M. H. "Plutonium Metabolism and Therapy." Department of Biological Sciences, Northern Illinois University, DeKalb, IL, March 2, 1978.
- Bhattacharyya, M. H. "Demonstration of a Direct Chelation Mechanism for DTPA-induced Excretion of Pu into Rat Bile." 26th Annual Radiation Research Society Meeting, Toronto, Canada, May 14-18, 1978.
- Bhattacharyya, M. H. "Internal Emitters." Department of Biological Sciences, Northern Illinois University, DeKalb, IL, November 6, 1978.
- Brown, M. S. "Action Spectrum for the Induction of Photoreactivable Events in a Double Mutant of *Escherichia coli* K12." 78th Annual Meeting of the American Society for Microbiology, Las Vegas, NV, May 14-19, 1978.
- Brown, M. S. "Action Spectrum for the Protection Against Far-Ultraviolet Lethality by Quinacrine." 78th Annual Meeting of the American Society for Microbiology, Las Vegas, NV, May 14-19, 1978.
- Buchholz, D. M. "Early Lymphocyte Responses in Radiation-induced Canine Leukemia." Mid-West Autumn Immunology Conference, Detroit, MI, November 6-7, 1978.
- Chan, E. W. "The Search for Onco-RNA-Viruses." Northwest Center for Medical Education, Indiana University School of Medicine, Gary, IN, February 3, 1978.
- Chan, E. W. "The Search for Onco-RNA-Viruses." Cancer Research Laboratory, University of Western Ontario, London, Ontario, Canada, February 21, 1978.
- Curtiss, J. R. B. "Methodology for the Assessment of Human Health Effects from the Coal Fuel Cycle." Seminar, Department of Energy Staff, Washington, DC, June 19, 1978.

- Danyluk, S. S. "Conformational Properties of Nucleic Acids in Solution: A Reexamination of Glycosyl Torsion Angles." Biomolecular Symposium, Madras, India, December 30, 1977.
- Danyluk, S. S. "Applications of Nuclear Magnetic Resonance Spectroscopy in Biological Structure Determinations." Madras Winter School, Madras, India, January 2, 1978.
- Danyluk, S. S. "Conformational Analysis of Oligonucleotide Structures." State University of New York, Albany, NY, February 23, 1978.
- Danyluk, S. S. "Investigation of Glycosyl Torsion Angles in Model Pyrimidine Nucleosides: Variation in Spin-Lattice Relaxation Times." Joint Biophysical Society-American Physical Society Meeting, Washington, DC, March 27-30, 1978.
- Danyluk, S. S. "Methylation Effects on Nucleic Acid Conformations." 11th Jerusalem Symposium, Tel Aviv, Israel, March 31, 1978.
- Danyluk, S. S. "Aspects of Nucleic Acid Conformations." Centre d'Etude Nucleaires de Saclay, Gif-sur-Yvette, France, March 31-April 14, 1978. (Also included in this trip were seminars in Israel and England).
- Danyluk, S. S. "Conformational Studies of Oligonucleotides in Solution." 61st Canadian Chemical Conference and Exhibition, Winnipeg, Manitoba, Canada, June 4-7, 1978.
- Dixon-Davis, D. "Standardization of Mortality for Variation in Socioeconomic Status." 11th Annual Meeting of the Society for Epidemiological Research, Iowa City, IA, June 14-16, 1978.
- Edwards, J. J. "Mapping of Human Red Cell Lysate Proteins by Two-Dimensional Electrophoresis." Federation of American Societies for Experimental Biology Annual Meeting, Atlantic City, NJ, April 9-14, 1978.
- Ehret, C. F. "The Chronopharmacology of L-DOPA: Implications for Orthochronal Therapy in the Prevention of Circadian Dyschronism." International Symposium on Clinical Chronopharmacology, Chronotherapeutics, and Chronopharmacy, Tallahassee, FL, February 10, 1978.
- Ehret, C. F. "Circadian Regulation: Basic Mechanisms, and the Role of Nutritional Factors and of Drugs as Environmental Zeitgebers." Department of Biological Sciences, De Paul University, Chicago, IL, February 24, 1978.
- Ehret, C. F. "The Circadian Connection to Regulatory Physiology and to Orthochronal Medicine." Department of Zoology and Physiology, Louisiana State University, Baton Rouge, LA, March 2, 1978.
- Ehret, C. F. "The Influence of Nutrition and Drugs on Circadian Regulation in Mammals: Implications for the Control of Jet Lag in Transmeridianal Travel." Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, DC, June 20, 1978.

- Ehret, C. F. "Molecular Mechanisms in Circadian Regulation." First Gordon Research Conference in Chronobiology, Plymouth, NH, July 3-7, 1978.
- Ehret, C. F. "Circadian Rhythm Research on the Effects of ELF Electric Fields on Small Rodents." USA-USSR International Effort, Department of Energy, Washington, DC, July 14, 1978.
- Ehret, C. F. "The Importance of Fundamental Research in Chronobiology to Human Health." National Conference on Health Research Principles, National Institutes of Health, Bethesda, MD, October 3-4, 1978.
- Ehret, C. F. "Exposure and Data Collection Facilities for Circadian Studies of Electric Field Effects upon Behavior, Thermoregulation, and Metabolism in Small Rodents." 18th Annual Hanford Life Sciences Symposium, Richland, WA, October 16-18, 1978.
- Ehret, C. F. "The Role of Diet in Phase Shifting of Circadian Rhythms during Jet Travel--Studies with Experimental Animals and with Humans." Department of Food Sciences and Nutrition, University of Minnesota, St. Paul, MN, October 23, 1978.
- Ehret, C. F. "Elements of Circadian Regulation." Department of Botany, University of Minnesota, St. Paul, MN, October 24, 1978.
- Elkind, M. M. "Cellular and Molecular Processes in Radiation Lethality." Department of Statistics, University of California, Berkeley, CA, January 16, 1978.
- Elkind, M. M. "Sublethal Damage and Potentially Lethal Damage, Are They Connected?" Lawrence Berkeley Laboratory, University of California, Berkeley, CA, January 17, 1978.
- Elkind, M. M. "DNA Damage and Mammalian Cell Killing." ICN-UCLA Symposium on DNA Repair Mechanisms, Keystone, CO, February 19-24, 1978.
- Elkind, M. M. "Molecular and Cellular Radiation Damage-Repair Processes." Loyola University Medical Center, Maywood, IL, March 8, 1978.
- Elkind, M. M. "Carcinogen + 'Sunlight' Interactions: Combined Effects of 7,12-Dimethylbenz(a)anthracene and Near Ultraviolet Light on Cultured Mammalian Cells." Ben May Laboratory, The University of Chicago, Chicago, IL, April 18, 1978.
- Elkind, M. M. "Implications of the Initial Portion of the Survival Curve for Low Dose Rate Teletherapy." International Cancer Research Workshop on Low Dose Rate Teletherapy, Milwaukee, WI, May 4-5, 1978.
- Elkind, M. M. "In Vitro Neoplastic Transformation: Its Dependence on Radiation Quality and Repair Processes." Eric Hall Symposium, 26th Annual Radiation Research Society Meeting, Toronto, Canada, May 14-18, 1978.
- Elkind, M. M. "Radiation Induced in Vitro Neoplastic Transformation and Its Relationship to Repair Processes." Armed Forces Radiobiology Research Institute, Bethesda, MD, June 26, 1978.

- Elkind, M. M. "Photosensitized Cytotoxicity by Polycyclic Aromatic Hydrocarbons and Coal-burning Effluents in Cultured Mammalian Cells." Division of Biomedical and Environmental Research, Department of Energy Contractor Workshop, Boca Raton, FL, November 11-14, 1978.
- Finkel, M. P. "Bone Cancer Induction by Radiation and Virus." Symposium on the Cancer Problem, American Association for the Advancement of Science, Houston, TX, December 26, 1978.
- Fritz, T. E. "Dose and Dose Rate Effects of Whole-Body Protracted Irradiation." Department of Biology, Harvard University, Cambridge, MA, February 22, 1978.
- Fritz, T. E. "Early and Late Effects of Continuous Low Level Radiation Exposure." Annual Meeting of the European Late Effects Project Group, Ulm, Germany, March 1, 1978.
- Fritz, T. E. "Relationship of Dose Rate and Total Dose to Responses of Continuously Irradiated Beagles." IAEA International Symposium on the Late Biological Effects of Ionizing Radiation, Vienna, Austria, March 13-17, 1978.
- Fritz, T. E. "Continuous Whole-Body Irradiation: Myelogenous Leukemia and Other Dose and Dose Rate Effects." Donner Laboratory, University of California, Berkeley, CA, April 19, 1978.
- Grahn, D. "Analytical Approaches to and Interpretations of Data on Time, Rate and Cause of Death of Mice Exposed to External Gamma Irradiation." IAEA International Symposium on the Late Biological Effects of Ionizing Radiation, Vienna, Austria, March 13-17, 1978.
- Grahn, D. "Genetic Effects of ^{239}Pu ." Radiobiology Laboratory, Medical Research Council, Harwell, England, March 30, 1978.
- Grahn, D. "Genetic and Cytogenetic Effects of Gonadal Burdens of Plutonium." Department of Biology, Northern Michigan University, Marquette, MI, April 24-25, 1978.
- Grahn, D. "Cytogenetic Damage in Spermatogonia of Mice from ^{239}Pu , External Fission Neutrons, and ^{60}Co Gamma Radiations." 26th Annual Radiation Research Society Meeting, Toronto, Canada, May 14-18, 1978.
- Grahn, D. "COSPAR's Reorganization." Committee on Space Research, Innsbruck, Austria, June 6, 1978.
- Grahn, D. "Health Aspects of Nuclear Electric Power." Redeemer Lutheran Church Men's Group, Elmhurst, IL, October 16, 1978.
- Grahn, D. "Genetic Effects of Radiation." Department of Biological Sciences, Northern Illinois University, DeKalb, IL, October 30, 1978.
- Hachey, D. L. "High Precision Metal Isotope Ratio Measurements Using Volatile Metal Chelates and Chemical Ionization Mass Spectrometry." 3rd International Conference on Stable Isotopes, Oak Brook, IL, May 23-26, 1978.

- Hachey, D. L. "Stereoselective Disposition of R-(-) and S-(+)-Methadone in Man." 3rd International Conference on Stable Isotopes, Oak Brook, IL, May 23-26, 1978.
- Hagan, M. P. "DNA Maturation in Synchronized Chinese Hamster Cells." Joint Biophysical Society-American Physical Society Meeting, Washington, DC, March 27-30, 1978.
- Han, A. "Repair of Radiation Induced Damage in Mammalian Cells." University of Health Sciences, Chicago Medical School, Chicago, IL, February 3, 1978.
- Han, A. "Cell Cycle Dependent Radiation Responses of Mammalian Cells." VA Hospital, Hines, IL, February 10, 1978.
- Han, A. "Radiobiological Characteristics of Neutron Beams in Chicagoland Area." American Association of Physicists in Medicine, Evanston, IL, April 8, 1978.
- Han, A. "Neutron RBE of Neoplastic Transformation in Vitro and the Effects of Dose Fractionation." 3rd Meeting on Fundamental and Practical Aspects of the Application of Fast Neutrons and Other High-LET Particles in Clinical Radiotherapy, The Hague, The Netherlands, September 12-16, 1978.
- Hass, B. S. "Comparative Mutagenicities of Coal Combustion Sources." Division of Biomedical and Environmental Research, Department of Energy Contractor Workshop, Boca Raton, FL, November 11-14, 1978.
- Kaspar, L. V. "Effects of Endotoxin Stress on Cellular and Humoral Responses in Dogs Exposed to Chronic Gamma Irradiation." 7th Annual Conference of the International Society for Experimental Hematology, Chicago, IL, August 27-31, 1978.
- Klein, P. D. "A Fecal ^{13}C Measurement for Quantitating Intestinal Malabsorption." 26th Annual Conference on Mass Spectrometry and Allied Topics, St. Louis, MO, May 28-June 2, 1978.
- Klein, P. D. "A Stable Isotope Menu--A la Carte or Price Fixe." Human Nutrition Committee, Department of Medicine, The University of Chicago, Chicago, IL, September 6, 1978.
- Klein, P. D. "The Use of ^{13}C Isotope Ratio Measurement in Clinical Research and Diagnosis." Japanese Society for Medical Mass Spectrometry, Tokyo, Japan, November 10, 1978.
- Klein, P. D. "Stable Isotopes in Clinical Research." Kyushu University, Fukuoka, Japan, November 13, 1978.
- Klein, P. D. "Some Recent Developments in Stable Isotope Applications." Kinki University, Osaka, Japan, November 14, 1978.
- Klein, P. D. "Some Recent Developments in Stable Isotope Applications." Stable Isotope Committee of Japan, Tokyo, Japan, November 15, 1978.

- Kubitschek, H. E. "DNA Lesions, Repair, Mutation, and Cancer." Department of Biology, Wheaton College, Wheaton, IL, January 13, 1978.
- Kubitschek, H. E. "Cell Growth: Exponential or Linear?" Departments of Physics and Microbiology, Clemson University, Clemson, SC, November 9, 1978.
- Lundy, R. T. "A Model for the Analysis of the Comparative Effects of Energy-related Effluents on Human Health." Population Association of America Meeting, Atlanta, GA, April 13-15, 1978.
- Lundy, R. T. "Methodology for Projecting the Health Impact of Air Pollution." Seminar, Department of Energy Staff, Washington, DC, June 19, 1978.
- Matsushita, T. "Cytometric and Correndonuclease Analysis of BSA-Protected Mouse Myeloma Cells." ICN-UCLA Symposium on DNA Repair Mechanisms, Keystone, CO, February 19-24, 1978.
- Matsushita, T. "Bacterial DNA Replication." Department of Biology, Wheaton College, Wheaton, IL, October 4, 1978.
- Matsushita, T. "Recombinant DNA Research." Department of Biology, George Williams College, Downers Grove, IL, October 18, 1978.
- O'Connor, T. E. "Cancer Virology." University of Illinois, Champaign, IL, February 8-9, 1978.
- Peak, M. J. "A Study of UV Reactivation of Ultraviolet Treated Transforming DNA by Competent Bacillus subtilis Cells." 22nd Wind River Conference on Genetic Exchange, Philadelphia, PA, June 9-13, 1978.
- Peak, M. J. "Inactivation of Transforming DNA by Far- and Near-Ultraviolet Light." International Workshop-Meeting on Solar Radiation Damage and the Induction of Skin Cancer, Lausanne, Switzerland, September 26-29, 1978.
- Peraino, C. "Detection of Tumorigenic Potential in Energy-related Pollutants." Division of Biomedical and Environmental Research, Department of Energy Contractor Workshop, Boca Raton, FL, November 11-14, 1978.
- Rahman, Y. E. "Potential of Liposomes in Metal Poisonings and Metal Storage Diseases." Symposium on the Potential of Liposomes as Drug Carriers, Geneva, Switzerland, March 2-3, 1978.
- Rahman, Y. E. "Liposomes Containing Desferrioxamine (DF) and Rhodotorulic Acid (RA) in Experimental Hemochromatosis." 21st Meeting of the American Society of Hematology, New Orleans, LA, December 2-5, 1978.
- Russell, J. J. "Comparison of Plutonium Distribution and Radiation Dose Received by Mouse and Dog Testis." 23rd Annual Meeting of the Health Physics Society, Minneapolis, MN, June 18-23, 1978.
- Russell, J. J. "Effect of Early and Delayed DTPA Therapy on Retention of Plutonium Citrate in Mouse and Dog Testis." 23rd Annual Meeting of the Health Physics Society, Minneapolis, MN, June 18-23, 1978.

- Ryu, E. K. "The Synthesis and Biological Activity of New Membrane-Targeted Molecular Depots of Nucleoside Analogues." Midwest Regional Meeting of the American Chemical Society, Fayetteville, AR, October 26-27, 1978.
- Sacher, G. A. "The Present Situation in Biogerontology." Kansas State University, Manhattan, KS, February 8-9, 1978.
- Sacher, G. A. "The Quadratic Low-LET Dose-Effect Relation for Life Shortening in Mammals: Implications for the Assessment of the Low-Dose Hazard to Human Populations." IAEA International Symposium on the Late Biological Effects of Ionizing Radiation, Vienna, Austria, March 13-17, 1978.
- Sacher, G. A. "The Role of Gerontology in Geriatric Education." National Academy of Sciences-Institute of Medicine, Washington, DC, March 20, 1978.
- Sacher, G. A. "Why Does Peromyscus Live Twice as Long as Mus?" Michigan State University, East Lansing, MI, April 2-3, 1978.
- Sacher, G. A. "Genetic Relation of Life-Span to Metabolic Rate for Five Mouse Strains and Their Hybrids." Federation of American Societies for Experimental Biology, Atlantic City, NJ, April 9-14, 1978.
- Sacher, G. A. "Biological Gerontology." Kansas State University, Manhattan, KS, May 31, 1978.
- Sacher, G. A. "Aging or Longevity: The Central Dialectic of Gerontology." University of Washington Institute on Aging, Seattle, WA, June 12, 1978.
- Sacher, G. A. "Mammalian Life Histories: Their Evolution and Molecular-Genetic Mechanisms." Given Institute of Pathobiology, Aspen, CO, July 29-August 4, 1978.
- Sacher, G. A. "Comparisons of Life Shortening in Different Mammalian Species Given Low-LET Radiation." XIth International Congress of Gerontology, Tokyo, Japan, August 20-25, 1978.
- Sacher, G. A. "Physiological and Molecular Factors in Lifespan Differences within and between Rodent Species." XIth International Congress of Gerontology, Tokyo, Japan, August 20-25,, 1978.
- Sacher, G. A. "Evolutionary-Genetic Factors Governing Differences in the Rate-of-Aging and Vulnerability Factors between and within Species." Symposium on Perspectives in the Modification of the Rate of Aging, Kyoto, Japan, August 30-September 1, 1978.
- Sacher, G. A. "The Constitutional Basis of Longevity in the Cetacea: Do the Whales and the Terrestrial Mammals Obey the Same Laws?" International Conference on Determining Age in Odontocete Cetaceans, La Jolla, CA, September 5-7, 1978.
- Sacher, G. A. "Unmet Needs of the Aging Population." Geriatric Workshop, Ohio State University, Columbus, OH, September 14, 1978.

- Sacher, G. A. "Contribution of Gerontology to Geriatric Training." Ohio Network of Consultants in Aging, Akron, OH, September 18, 1978.
- Sacher, G. A. "Physical Activity and Its Role in Aging." University of Illinois Aging Program Retreat, Harvey, IL, October 16, 1978.
- Sacher, G. A. "The Evolution of Longevity." Dalhousie University, Halifax, Nova Scotia, November 2, 1978.
- Sanderson, M. H. "The Effect of Daily Prenatal Gamma Irradiation on Ovarian Cortex of Beagle Puppies." 9th International Congress of Electron Microscopy, Toronto, Canada, August 1-9, 1978.
- Seed, T. M. "Hemopathological Consequences of Protracted Gamma Irradiation in the Beagle: Pre-Clinical Phases of Leukemia Induction." IAEA International Symposium on the Late Biological Effects of Ionizing Radiation, Vienna, Austria, March 13-17, 1978.
- Seed, T. M. "The Biology of Malaria." Department of Biology, St. Francis College, Joliet, IL, May 2, 1978.
- Stearner, S. P. "Cardiovascular Degeneration in the Aging, Irradiated Mouse." AAAS Annual Meeting, Washington, DC, February 12-17, 1978.
- Stearner, S. P. "Comparative Effects of Fission Spectrum Neutrons and ^{60}Co Gamma Rays on Long-Term Cardiovascular Changes." 26th Annual Radiation Research Society Meeting, Toronto, Canada, May 14-18, 1978.
- Stellaard, F. "Identification of Abnormal Bile Acids in Disease States and Their Detection by Gas Chromatography-Mass Spectrometry-Computer Techniques." Vth Bile Acid Meeting, Freiburg, Germany, June 12-13, 1978.
- Stellaard, F. "Identification of Atypical Bile Acids by Gas Chromatography/Mass Spectrometry/Computer. 5th International Symposium in Biochemistry and Medicine, Rimini, Italy, June 19-21, 1978.
- Suehiro, M. "Investigation of an Apparent Isotope Effect between $^{13}\text{CO}_2$ and $^{14}\text{CO}_2$ in Trioctanoin Breath Test." 3rd International Conference on Stable Isotopes, Oak Brook, IL, May 23-26, 1978.
- Szczepanik-Van Leeuwen, P. A. "Identification of Abnormal Bile Acids in Disease States and Their Detection by Gas Chromatography-Mass Spectrometry-Computer Techniques." 26th Falk Symposium, Vth Bile Acid Meeting, Freiburg, Germany, June 13-14, 1978.
- Tewari, R. "Theoretical Complications of Conformational Preferences in Dinucleoside Monophosphates. Implications for Recognition by Exonucleases." Joint Biophysical Society-American Physical Society Meeting, Washington, DC, March 27-30, 1978.
- Tewari, R. "Conformational Preferences in Dinucleoside Diphosphates: Influence of Charge." 11th Midwest Theoretical Chemistry Conference, Columbus, OH, May 18-20, 1978.

- Tewari, R. "Theoretical Calculations of Conformational Preferences in Dinucleoside Triphosphates." American Conference on Theoretical Chemistry, Boulder, CO, June 25-30, 1978.
- Tolle, D. V. "Irradiation-induced Canine Leukemia--A Proposed New Model. Incidence and Hematopathology." 7th Annual Conference of the International Society for Experimental Hematology, Chicago, IL, August 27-31, 1978.
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